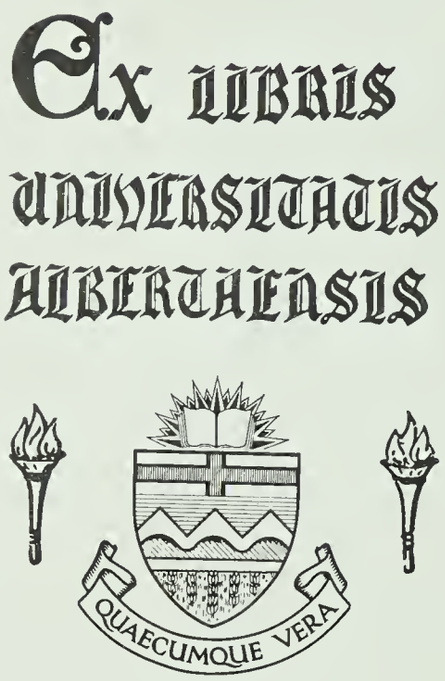

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THE UNIVERSITY OF ALBERTA

PHYSIOLOGICAL CHANGES IN SHEEP DURING EATING

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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DEPARTMENT OF ANIMAL SCIENCE

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FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Physiological Changes in Sheep During Eating", submitted by Rusins Berzins, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Experiments were conducted with seven sheep to study short term changes in thyroid gland secretion, blood composition, and metabolic activity during eating. Isotope trials with sheep previously injected with I^{131}, showed a 10% decrease in thyroid gland radioactivity during eating. Measurements of I^{131} activity in jugular and thyroid venous blood samples revealed a 5% decrease during eating and indicated that activity in jugular blood was consistently lower than that in thyroid venous blood. Also, jugular blood flow increased by 80% during the first 15 min of feeding. From these trials, it was concluded that thyroid hormone secretion was enhanced during eating. Changes in appetite after thyroidectomy and during thyroxine replacement therapy overshadowed any changes in cardiac and metabolic responses which might have been altered by such treatments.

Analyses of arterial and venous blood showed consistent changes in blood metabolite balances and packed cell volumes. Hematocrits increased 25% over pre-feeding values during the first minute of eating, but plasma glucose and FFA concentrations decreased steadily throughout the feeding period. From the results of jugular plasma insulin assays, it was concluded that the hypoglycemic response during eating was not mediated by insulin. Plasma protein concentrations increased by 12% during eating and appeared to coincide with elevated salivary and gastro-intestinal secretion rates. The elevation of venous plasma protein-bound-iodide concentrations during eating might have resulted from decreases in plasma volume. Venous pH, and pO_2

decreased, while pCO_2 increased rapidly at the onset of eating.

During beta-adrenergic blockade with propranolol, the initial peak in blood pressure, which occurred upon presentation of food, was abolished. However, the slight increase in mean arterial pressure, which persisted for the first 10 min of feeding, was unaffected.

Atropine-induced parasympathetic blockade (0.05 and 0.1 mg/kg atropine), challenged with periodic injections of acetylcholine, tested by direct vagal nerve stimulation, and judged by changes in heart rate and reticulo-rumen motility, appeared effective for 20 min. The presence of an atropine-degrading enzyme in sheep was suggested.

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INTRODUCTION

Rapid changes in the cardiac and metabolic functions of sheep have been noted during eating and the involvement of definite control systems has been suggested from the pattern of these responses. The parasympathetic nervous system is known to regulate many secretory and muscular activities during feeding, but has not been shown to induce tachycardia during eating. The sympathetic portion of the autonomic nervous system is known to affect calorigenesis and induce tachycardia under a variety of conditions, but its role in regulating the eating response has been shown to be limited.

The purpose of this work was to obtain more information about physiological changes during eating and to identify the mechanisms controlling heart rate and oxygen consumption. Experiments were designed to measure changes in blood constituents and to study the extent of autonomic and hormonal control in the regulation of cardiac and metabolic activity in sheep during eating.

REVIEW OF LITERATURE

A study of physiological changes that occur in sheep during eating is of significance to animal production in view of the proportion of time that ruminants spend feeding. Results of studies of the grazing behaviour of domestic ruminants indicate that sheep may graze a good pasture for as long as 8 to 14 hours per day (England, 1954). That increased energy was expended during eating was noted by Ustjanzew (1911).

Recent estimates of the energy expended by sheep during eating demonstrate increases over resting oxygen consumption ranging from 50-70 per cent (Blaxter and Joyce, 1963; Young, 1966; Webster and Hays, 1968). Elevated heart rates during feeding have been reported for sheep (Blaxter, 1948; Webster, 1966) and cattle (Kelley and Rupel, 1937; Blaxter, 1943). It is well known that a relationship exists between heart rate and oxygen consumption, or metabolic rate, for man and animals (Henderson and Prince, 1914; Brody, 1945). Several workers have used this relationship as a basis for estimating oxygen consumption of men exposed to a variety of conditions (Read, 1924; Lundgren, 1946; Malhotra, Sen Gupta and Rai, 1963). A similar correlation exists for ruminants under resting conditions (Blaxter, 1948; Blaxter and Wood, 1951). Webster (1967) demonstrated a close relationship between heart rate and energy expenditure, when energy expenditure was increased by cold exposure, or by increased levels of food intake. He also suggested that measurements of heart rate during eating continued to reflect the metabolic rate. According to Young (1967) however, heart rate cannot be used as an accurate index of energy expenditure during

the feeding period because of the large standard error associated with individual regression equations.

The impetus for these metabolic transitions appears to be generated at various sites. Increased salivary secretion (Bailey, 1961; Bailey and Balch, 1961; Meyer et al., 1964), reticulo-rumen motility (Christopherson, 1967) and the muscular activities of prehension and mastication are major items in the energy cost of feeding as reflected by oxygen consumption (Young, 1966).

Stacy and Brook (1965) observed consistent increases in plasma osmotic pressure of sheep after the start of feeding and were able to isolate an antidiuretic material from the post-prandial urine. A successful biological assay of this compound in water-loaded sheep enabled them to conclude that pen-fed sheep regularly experience conditions that classically stimulate the neurohypophysis to control the renal excretion of water by secreting antidiuretic hormone (ADH). As a result, feeding is accompanied by urine secretion of increased osmolality and decreased volume. Brook and Blair-West (1968) measured increases in sheep plasma renin concentration from less than 1 ng/ml per hr to 2-4 ng/ml per hr within 30-60 minutes of the start of feeding and suggested the involvement of the renin-angiotensin system in regulating renal activity during feeding.

Coincident with an increased metabolic rate during eating, there is a significant elevation of deep body temperature. Mendel and Raghavan (1964) recorded increased carotid and jugular blood temperatures upon feeding, while Baker and Hayward (1968) measured elevated temperatures in the carotid rete and circle of Willis in

sheep in response to the sight of food. Upon feeding, intracranial temperature remained elevated and central arterial temperature rose steadily. Findlay and Ingram (1961) observed similar changes in central body temperature of steers during feeding.

Sellers et al, (1964) detected a 25 to 30% rise in blood flow through the right ruminal and omasal arteries when sheep were fed. Fronek (1968), using dogs, observed increased heart rate, blood pressure, cardiac output, and brachiocephalic flow during ingestion which he attributed to sympathetic nervous control. As the feeding period lasted only three minutes, these effects may be analogous to transient changes observed in sheep upon the presentation of food. These transient changes include the immediate increase in heart rate and oxygen consumption, noted by Blaxter and Joyce (1963) when tracheostomized sheep were fed, or were sham-fed, and were attributed to excitement (Webster, 1966).

Sympathetic nervous activity was examined by Hays (1968) who studied the effects of propranolol on tachycardia and oxygen consumption in sheep during eating. He observed a 15% decrease in peak heart rate but no reduction of metabolic rate during beta adrenergic blockade, and concluded that the sympathetic nervous system, acting through the beta receptors, is of minor importance in initiating and regulating the changes of these phenomena caused by eating. Similar degrees of cardiac adaption to increased energy expenditure during exercise have been noted in the absence of sympathetic-induced cardioacceleration (Epstein et al., 1965; Cumming and Carr, 1966; Cronin, 1967). Donald and Samueloff (1966) demonstrated

that propranolol was effective in blocking tachycardia in an isolated dog's heart perfused by the circulatory system of an intact, exercising dog. However, the heart rate of the exercising dog was not reduced by beta blockade. They concluded that there is an intrinsic mechanism in the dog's heart that brought about cardioacceleration in proportion to the amount of work performed. Webster and Hays (1968) implied a similar mechanism in sheep. This would suggest involvement of other systems, perhaps neural or hormonal, in the control of heart rate.

Increased parasympathetic nervous activity during eating has long been recognized; parasympathetic control of salivation and gut motility was well described by Dukes (1960). Parasympathetic regulation of heart rate occurs by a negative, or inhibitory, mechanism because upon vagal nerve stimulation a decreased heart rate is observed. As constant vagal tone is exerted on the heart, cardioacceleration, mediated by the parasympathetic system, can only be achieved by some degree of parasympathetic blockade, or reduction of vagal tone. Hays (1968) suggested possible parasympathetic control of heart rate in sheep during feeding from preliminary experiments in which sympathetic beta blockade did not appear to eliminate the increased heart rate of feeding sheep.

A hormonal basis for tachycardia and increased metabolic rate during feeding cannot be dismissed. One classic action of thyroid hormones is their effect on calorigenesis. This is reflected in increased oxygen consumption in the whole animal, or in isolated tissues *in vitro* (Ingbar and Woeber, 1968). This response to thyroxine occurred after a latent period of several hours, or days;

triiodothyronine (T_3) caused a more prompt effect of shorter duration. Guz, Kurland and Freedberg (1961) observed an increased heart rate in myxedematous rabbits 1-3 hours after injection of 200-400 µg T_3 and an increase in oxygen consumption 3.5 to 4 hours after injection. Decreased amounts of hepatic glycogen and increased amino acid incorporation (Tata et al., 1963) have been measured after T_3 administration to thyroidectomized rats. Mobilization of free fatty acids (FFA) in man by T_3 was reported by Rich, Beirman and Schwartz (1959). Although a latent period appears associated with thyroid action, the effects mediated by the thyroid hormones appear similar to cardiac and metabolic changes observed in sheep during feeding.

An implication of increased thyroid activity associated with eating was made by Nathanielsz (1967), who reported a diurnal rhythm in the disappearance of thyroxine from the blood of rats and calves. He found the half-life of labeled thyroxine to be shorter during the day only if animals were fed during the day. When food was withheld, the daytime rate of dissappearance of thyroxine decreased and was further exceeded by the nocturnal rate when food was offered at night.

Changes in protein-bound I^{131} (PBI^{131}) concentration in thyroid venous blood after both nervous and hormonal stimulation of the thyroid gland, were reported by Ishi, Shizume and Okinaka (1968). Stimulation of the nodose ganglion on either side increased thyroid vein PBI^{131} levels, within 15 minutes, in all dogs studied. Vagal stimulation increased both thyroid blood flow and PBI^{131}. They concluded that the response to neural stimulation appeared more

promptly, although it was less pronounced, than the response to thyrotropin (TSH).

The blood system is not the only carrier of thyroid secretions. Daniel, Plaskett and Pratt (1967) observed that lymph from the thyroid gland contained more T_4 than thyroid venous blood. Furthermore, the iodoprotein content of rat thyroid lymph was raised sharply by giving TSH, and also by thyroid massage, which expressed the lymph contained within the lymphatics of the thyroid gland (Daniel, Pratt, et al, 1967).

The thyroid hormones are by no means unique in their effects on metabolic and cardiac function. The kinins, a group of short-chain polypeptides, are capable of producing profound vasodilation, systemic hypertension, an increase in salivation, lacrimation, and cardiac output, as well as edema and tachycardia (Melmon, 1968). Bradykinin, a nonapeptide and kallidin (lysyl-bradykinin) are derived from α_2-globulin (kininogen) by a group of highly specific, endogenous, proteolytic enzymes, known as kallikreins, that occur in urine, saliva, pancreatic secretion, blood and other body fluids. Blood contains the inactive enzyme kallikreininogen which can be rapidly converted to the active form, kallikrein, by a variety of factors, including changes in blood pH, that disturb plasma equilibrium (Margolis, 1963; Schacter, 1964). Adrenaline and sympathetic discharge may also liberate kallikrein (Melmon, 1968). Although the kinins are short-lived in blood (Erdös, 1963), they may function in mediating physiological changes in sheep during eating.

Alternatively, 5-hydroxytryptamine (5-HT), which stimulates

a variety of smooth muscle and nerves, exhibits marked effects on the cardiovascular, respiratory, and gastrointestinal systems. Depending on the dose, route of administration, anesthetic, neurogenic constrictor tone, general conditions of the cardiovascular system, and especially the species studied, injection of 5-HT may cause blood pressure to rise, or fall, or show a polyphasic change (Page, 1954). This complexity arises from the fact that 5-HT influences the circulation through a number of partially antagonistic mechanisms, including the reflex blood pressure and heart rate controls. Although some species variation exists, by far the greatest proportion of 5-HT originates from the alimentary tract, while some is found in blood platelets and the brain (Douglas, 1965). Although the physiological role of 5-HT remains unclear, a possible function during eating cannot be overlooked.

Other possible mediators of physiological responses during eating are the prostaglandins, substance P, and adrenocortical hormones. The prostaglandins (prostanoic acids) are found in considerable quantities in the lungs, brain, kidneys, thymus, pancreas, and iris of various mammals (Bergström et al., 1963). Prostaglandins elevate heart rate and lower systemic vascular resistance (Carlson, 1967); they are removed from the circulation by the lungs (Ferreira and Vane, 1967), and are actively metabolized by lung tissue (Anggard and Samuelsson, 1967). But the biological functions of the prostaglandins are still unresolved.

Another biologically active polypeptide, substance P, may be involved in the eating response. It is present in relatively high

concentrations in the gastrointestinal tract, appears to be a more powerful vasodilator than bradykinin (von Euler and Gaddum, 1931), and is a strong stimulator of the human gut (Liljedahl, Mattsson, Pernow, 1958).

Possible involvement of adrenal cortex hormones could be postulated from three lines of evidence: Stacy and Brook (1965) confirmed the involvement of ADH in the control of water excretion in sheep during eating; Gwinup et al. (1968) proposed the theory that ADH induces ACTH secretion which in turn stimulates hormone release from the adrenal cortex; Brook and Blair-West (1968) suggested the involvement of the renin-angiotensin system during feeding, while Genest et al. (1960), and Laragh et al. (1960) demonstrated that angiotensin had specific stimulant effects on aldosterone secretion.

Although no positive conclusions may be formed from this review, the possible involvement of the aforementioned systems must be considered when postulating the mechanisms which control heart rate and catalyze metabolic transitions during eating.

EXPERIMENTAL

A. Methods and Materials

1. Animals and rations

Two Lincoln rams (5U, 23V) and five wethers of mixed breeding, (135T, 244T, 129U, 168U, and Gus) ranging in weight from 90-120 kg, were housed in a thermoneutral environment (Blaxter, 1962) in individual feeding crates. All sheep received 500 g alfalfa-brome hay twice daily. Cobalt-iodized salt and water were available at all times.

2. Label preparation and measurement

Sodium iodide (I^{131})[1] in isotonic buffered solution containing sodium thiosulfate, pH 7-8, activity 1 mc per ml, was diluted 1:20 with 0.9% w/v NaCl. Appropriate doses were injected subcutaneously (sc) in the groin region.

The radioactive decay of *in vivo* thyroidal I^{131} was measured as counts per minute (cpm) with a NaI(Tl) crystal scintillation detector (model DS-5)[2]. Cpm were recorded with an automatic scaler (model 192A)[2]. Total blood radioactivity was measured using a DS-5 detector with a XT2WO well type crystal[2].

Difficulties were encountered in maintaining constant positioning of the probe. Initially the probe was fixed on a stand and the animal's head was restrained with a halter. But when sufficient movement was permitted for the sheep to eat in comfort, the geometry of counting was altered. Then, to limit lateral movement of the thyroid,

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[1]The Radiochemical Centre, Amersham, England.

[2]Nuclear Chicago Corp., Des Plaines, Illinois.

wooden blocks were secured on either side of the neck; but forward movement could not be adequately restricted. Subsequently the probe was counterbalanced over two pulleys and suspended under the thyroid. The probe was fixed to the animal's neck with an adjustable collar which had a cylindrical opening, under the thyroid, made to receive the detector head (Fig. 3).

3. Physiological measurements

Heart rates were measured from electrocardiograms (ECG) recorded with a Sanborn physiological recorder and a high gain pre-amplifier (model 350-2700C)[3]. An oscilloscope (model S-55)[4] and stopwatch were used in some trials. Three surface electrodes, one behind each shoulder, equidistant from the humero-scapular joint and the third under the left elbow, all in the same vertical plane as the heart, were attached to the skin with a two-inch square of adhesive tape and rubber cement. Electrical contact was maintained with a water soluble transmission gel (Aquasonic 100)[5].

Oxygen consumption was determined from continuous measurement of respiratory exchange using the open circuit respiration apparatus of Webster and Hicks (1968). The sheep's head was enclosed in a respiration chamber, which sealed around the neck with an elastic cuff and allowed sufficient freedom of movement to eat in comfort. Food was placed in the hood before starting each experiment, but access to the feed was controlled by a removable false bottom in the hood.

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[3]Hewlett-Packard (Canada) Ltd., Montreal, Quebec.

[4]Paco Electronics, Glendale, L.I., N.Y.

[5]Parker Laboratories, Inc., Irvington, N.J.

This arrangement eliminated previously observed artefacts due to opening the door at the front of the hood.

According to Joyce and Blaxter (1964) the volume of expired air never exceeded 800 l/hr when a sheep was exposed to severe cold stress sufficient to elevate heat production by nearly five-fold. In the present experiments, ventilation rate was maintained at 3000 l/hr in an effort to minimize CO_2 accumulation and water condensation inside the respiration chamber.

Changes in energy expenditure were calculated from relative changes in O_2 consumption, where average pre-feeding O_2 consumption was set at 100%. Rectal temperature was measured by a telethermometer[6] with a thermistor probe kept in the rectum throughout the trial.

Blood pressure values were obtained from sheep with permanent carotid artery loops. Blood pressure was monitored with the Sanborn physiological recorder, a carrier preamplifier (model 350-1100C), a pressure transducer (model 267BC)[7], through an intramedic polyethylene catheter (PE-190/S36)[8] inserted into the common carotid artery and directed towards the heart. The catheter was flushed periodically with heparinized saline to prevent clot formation. Reticulo-rumen motility was measured by recording intra-ruminal and reticular pressure changes using the above mentioned pressure transducer, preamplifier and recorder. Pressure changes were detected by two weighted, water-filled balloons, placed into the rumen and

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[6]Yellow Springs Instrument Co., Yellow Springs, Ohio.

[7]Hewlett-Packard (Canada) Ltd., Montreal, Quebec.

[8]Clay-Adams Inc., New York, N.Y.

reticulum, and transmitted to the transducer by a continuous water column through Tygon[9] tubing. Insertion of the reticular balloon was facilitated by a length of copper tubing which extended from the balloon, through the fistula, and was connected to the flexible tubing. Positioning of the balloon was possible by bending the copper tubing to the desired shape before inserting.

4. Blood sampling and drug administration

A carotid artery - jugular vein loop with a functioning lobe of the thyroid gland was prepared in a five-year-old cross-bred wether (Falconer, 1963). A second loop containing only the jugular vein was made between the thyroid and the junction of the internal and external jugulars (Fig. 1). By cannulating the jugular vein within the loop, so that the catheter tip lay close to the junction of the thyroid vein, and by compressing the jugular above and below the thyroid gland, it was possible to collect thyroid venous blood.

All carotid artery and jugular vein catheterization was performed with intramedic polyethylene catheters (PE-190/S36) through 2 inch, 13 gauge needles. The sheep were catheterized at least one hour before any drug was administered or blood sampled. Novocain was injected subcutaneously when skin sutures were required to secure the ascending jugular catheter in the thyroid-containing loop. Other jugular and carotid catheters were secured with surgical gauze and adhesive tape. Blood was sampled with disposable plastic syringes, collected in heparinized polyethylene test tubes (0.2 ml heparin per tube) and immediately cooled in ice water. The catheter

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[9]U.S. Stoneware Inc., Akron, Ohio.

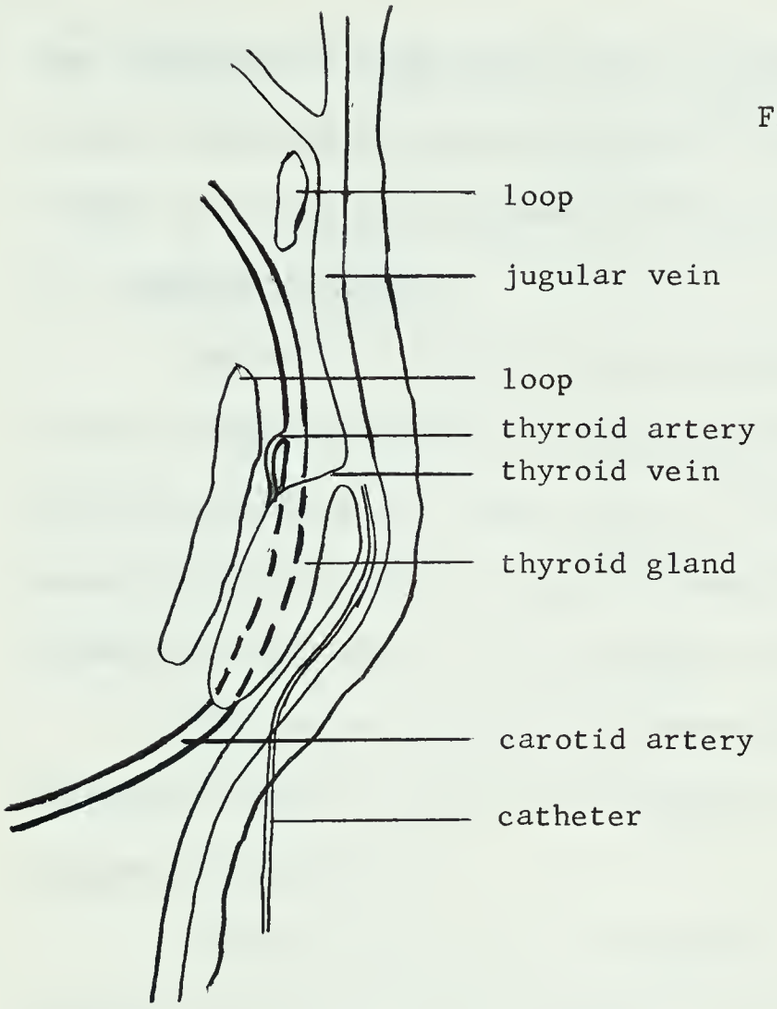

Figure 1. Diagram of exteriorized thyroid gland.

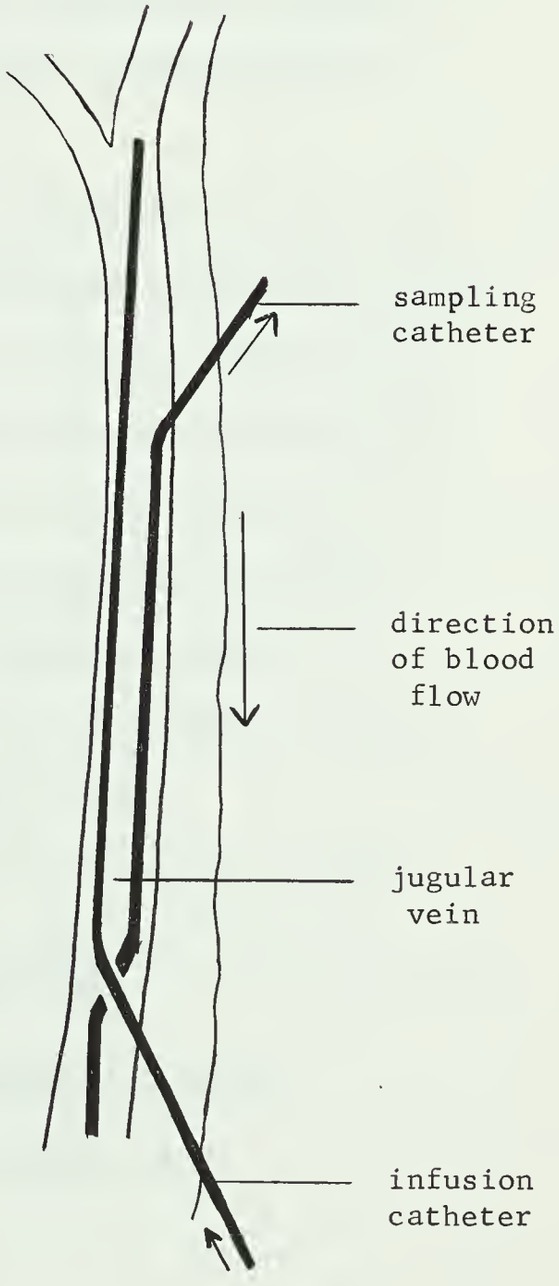

Figure 2. Method of jugular vein catheterization for blood sampling and dye infusion.

was flushed with heparinized saline between sampling. A variable speed infusion pump (series 600-950V)[10] was used for regulated intravenous (iv) drug administration (Table 1).

5. Analytical methods

Packed cell volumes were determined with heparinized capillary tubes (Fisher, #2-668-55), a micro-capillary centrifuge (model MB) and a micro-capillary reader (cat. no. 2201)[11]. The capillary tubes were filled and sealed at one end immediately after each sample was drawn from the animal, and thereafter centrifuged within 15 minutes.

The glucose content of protein-free preparations (Somogyi, 1945) of blood, or plasma was estimated using a glucose oxidase reagent (Glucostat)[12].

Plasma protein-bound iodide (PBI) concentrations were estimated by the Hycel Cuvette method[13]. The original procedure suggested the use of an ion-exchange resin to remove inorganic iodide from the unknown solutions. However, more consistent results were obtained by using 3.5% $HClO_4$ to precipitate PBI from solution (Steiner and Carpenter, 1962). This precipitate was then digested with $HClO_4$ and further processed as outlined in the original procedure.

Free fatty acid content of plasma was estimated by a photometric adaptation of Dole's method (Mosinger, 1965).

Total plasma protein was estimated by the method of Folin

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[10]Harvard Apparatus Co. Ltd., Dover, Mass.

[11]International Equipment Co., Needham Hts., Mass.

[12]Worthington Biochemical Corp., Freehold, N.J.

[13]Hycel, Inc., Houston, Texas.

Table 1

List of drugs used: Their dose, concentration, and route of administration.

Drug	Dose	Concentration	Mode of administration
Pentobarbital sodium[1] (Nembutal)	as required	60 mg/ml	iv
Heparin sodium[2]		10 mg/ml	iv
Propranolol[3] (ICI 45,520, Inderal)	0.5 mg/kg	2 mg/ml	iv
Atropine sulfate[4]	0.05 mg/kg	0.5 mg/ml	iv
Noradrenaline[5]	0.1 µg/kg/min		iv
Thyrotropin (TSH)[6]	1 µg/kg/min		iv
L-3,3',5-triiodothyronine[6]	1 µg/kg/min		iv
L-thyroxine (T_4)[6]	3 µg/kg/day	0.2 mg/ml	sc
Acetylcholine bromide[7]	10 µg/kg	1 mg/ml	iv
Sodium sulfobromophthalein[8] (bromsulphalein)	1.67 ml/min	3 mg/ml	iv

[1]Abbott Laboratories Ltd., Montreal Quebec.

[2]Riker Pharmaceutical Co. Ltd., Cooksville, Ontario.

[3]Ayerst Laboratories, St. Laurent, Quebec.

[4]British Drug House (Canada) Ltd., Toronto, Ontario.

[5]Winthrop Laboratories, Aurora, Ontario.

[6]Nutritional Biochemicals Corp., Cleveland, Ohio.

[7]Eastman Organic Chemicals, Rochester, N.Y.

[8]Hynson, Westcott and Dunning, Inc., Baltimore, Md.

and Ciocalteau (1927).

Plasma amino nitrogen determination followed the photometric procedure developed by Moore and Stein (1954).

Plasma insulin levels were measured by a double-antibody immuno-assay (Manns and Boda, 1967). Modifications to the method included the substitution of gelatin for bovine serum albumin and the use of I^{125} instead of I^{131}. The analyses were performed by Dr. J.G. Manns at the Western College of Veterinary Medicine in Saskatchewan.

Jugular vein blood flow was determined by bromsulphalein dye dilution as assessed from plasma optical density (OD) measurements (Shoemaker, 1960). The dye was infused through a retrograde, jugular catheter which terminated at the junction of the internal and external jugular veins; blood was sampled through another catheter from a point approximately 12 inches below the site of infusion. The retrograde catheter was inserted approximately 4 inches below the one used for sampling. The reason for having both catheters pass through a common portion of jugular vein was to promote dye mixing (see Fig. 2).

Blood pH, pO_2 and pCO_2 were measured with the Blood Micro System (Type BMS3), PHM71 acid base analyzer, PHA930 pO_2 module, and PHA931 pCO_2 module[14].

6. Statistical analyses

The statistical methods used to aid in the interpretation of these data were described by Steel and Torrie (1960).

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[14]Radiometer, Copenhagen, Denmark.

B. Experiment I Estimation of Sheep Thyroid Gland Activity During Eating

A series of trials was designed to estimate sheep thyroid gland activity to determine whether thyroid hormones are involved in the short term changes in metabolic and cardiac activity which occur during eating.

Trial 1 Estimation of thyroid secretion

Objective

As the metabolic and cardiac effects induced by thyroid hormones appear similar to those occurring in sheep during eating but have always been associated with a latent period of several hours or days, this trial was designed to measure short-term changes in thyroid secretion to determine whether thyroid hormones are involved in the eating response.

Experimental

Two wether sheep, 129U and 168U, were each given 300 μc I^{131} sc (approximately 4 μc/kg body weight), following which, neck counts were recorded for 15 days. The sheep were fed alfalfa-brome hay for 10 min periods during each 30-45 min counting session. During these sessions the animals were held in individual metabolism crates, haltered, and allowed only sufficient head movement to permit them to eat hay out of a small feed-box fixed to the front of the crate. The detector probe was placed on a stand and positioned directly under the thyroid gland, adjacent to the sheep's neck.

Results and Discussion

Counts per minute (cpm) were averaged for 10 min periods before, during, and after feeding, and were expressed as per cent of mean pre-feeding cpm (Table 2). However, this system was unsatisfactory since the geometry of counting could not be kept constant and changes in cpm similar to those measured during the trial could be obtained by merely holding the sheep's head in different positions relative to the detector. Therefore, a significant portion of the observed decrease in neck cpm during this eating trial may be attributed to forward movement of the sheep during prehension, which caused movement of the thyroid gland away from the detector.

Table 2

Changes in neck counts during 10 min periods expressed as per cent of mean pre-feeding cpm.

sheep	pre-feeding	during feeding	post-feeding
129U	100	61.4	92.9
129U	100	88.0	105.0
129U	100	91.1	104.0
129U	100	89.6	88.9
129U	100	91.0	97.2
129U	100	97.2	94.0
168U	100	70.0	101.0
168U	100	49.0	99.0
168U	100	91.0	92.5
168U	100	93.0	88.0
168U	100	99.0	96.0
168U	100	96.0	95.0
$\bar{X}$	100%	84.7%	96.1%
$S_{\bar{x}}$		4.5%	1.6%

$S_{\bar{x}}$ = standard error of mean
$\bar{X}$ = mean

Trial 2 Uptake and release of I^{131} by the thyroid gland

Objective

Results of Trial 1 suggested that the decrease in neck counts observed during eating was largely due to movement of the animal. Trial 2 was therefore designed to eliminate this artefact from neck count measurements and to provide an estimate of I^{131} uptake by the thyroid gland. I^{131} activity of jugular venous blood sampled during this trial was also measured.

Experimental

Two Lincoln rams (5U and 23V) were given 250 μc I^{131} sc and fed once per day. The feeding periods during counting were extended to 30 minutes. Immediately after counting, the sheep were fed the remainder of their daily ration. To minimize thyroid movement relative to the detector probe, the detector was counterbalanced and attached to the sheep's neck with a collar, as previously described (see Fig. 3 and Methods and Materials). The animal was held in an elevated crate and allowed free head movement during eating.

To estimate the proportion of I^{131} taken up by the thyroid, the natural decay of a 250 μc standard I^{131} sample was measured daily. A small bottle containing this mock thyroid sample was placed inside a short length of 1 inch (i.d.) tygon tubing which was then positioned in the counting collar at the approximate level of the thyroid gland (Fig. 3). The counts obtained for a standard in this system are shown in Fig. 4 and the rate of decay of activity of the material used is compared to the natural decay of I^{131}. The standard values for various dosages of label were taken from Fig. 4.

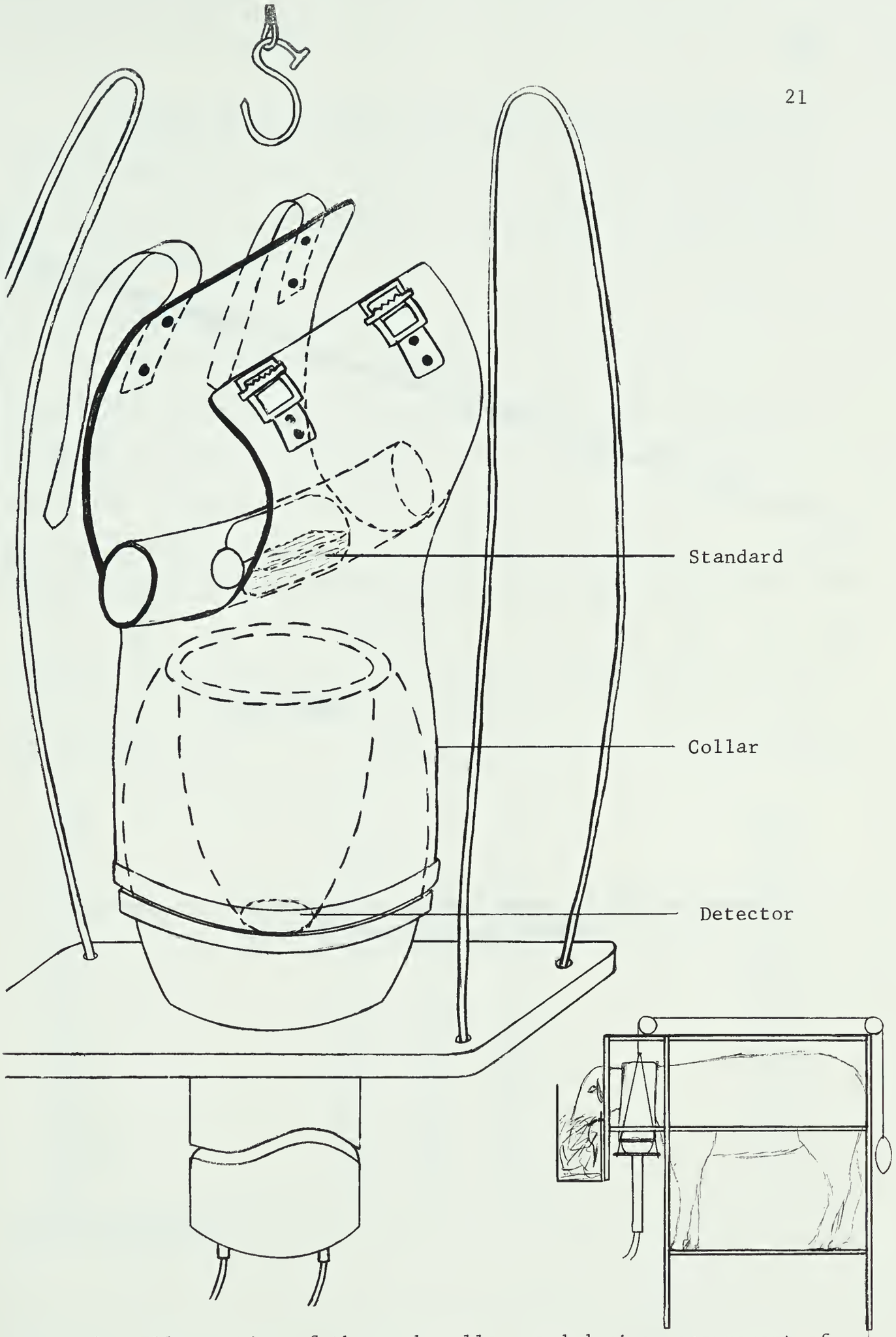


Figure 3. Illustration of the neck collar used during measurement of I^{131} activity in thyroid glands and standard samples.

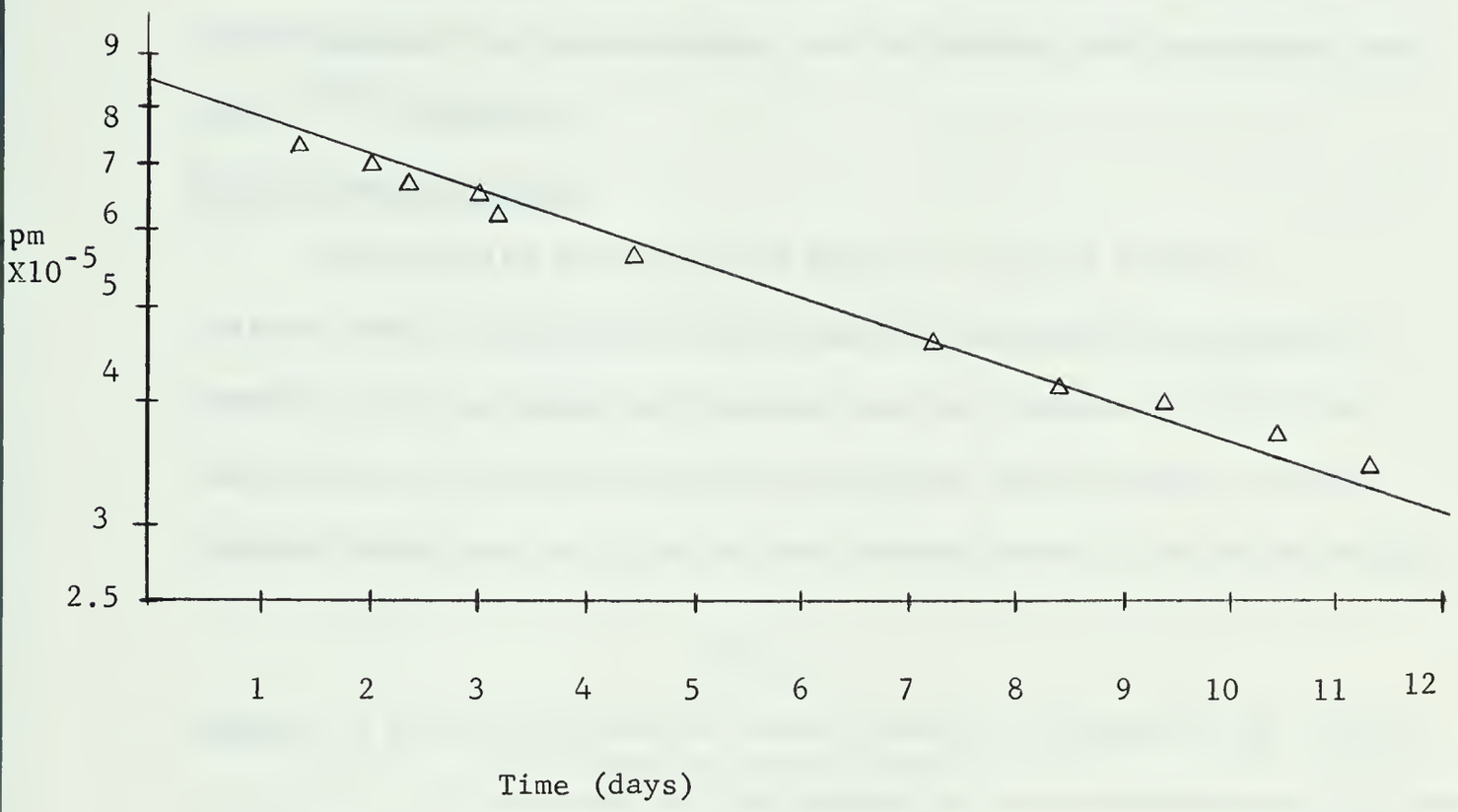


Figure 4. Comparison of natural decay of I^{131} to counts obtained from a 250 μc standard.

A series of 2 ml jugular vein blood samples were taken from sheep 5U and 23V at 2 to 3 min intervals during the feeding session, for measurement of radioactivity, on the seventh and succeeding days after I^{131} injection.

Results and Discussion

The results obtained from sheep 5U and 23V (Table 3) indicate that variability in neck counts, previously attributed to movement, was decreased and suggest that net release of I^{131} from the region of the thyroid gland was enhanced during eating. Post-feeding values were still below pre-feeding rates 10 min after eating.

Table 3

Changes in neck counts during 10 min periods expressed as per cent of mean pre-feeding cpm.

pre-feeding	feeding (min)			post-feeding
	0-10	11-20	21-30	
100	99.1	96.0	94.2	102.4
100	98.8	97.8	102.1	97.3
100	88.5	84.6	84.6	99.8
100	90.8	85.6	88.6	90.3
100	89.1	85.9	80.8	87.5
100	86.7	81.2	85.3	85.2
100	98.5	97.2	99.5	101.1
100	86.0	90.2	*	99.2
100	91.8	88.7	90.3	91.2
100	89.7	87.2	86.1	98.6
100	88.3	78.0	83.7	96.3
100	89.9	82.5	81.3	95.2
100	96.1	87.8	94.9	103.5
100	94.6	97.5	*	98.8
$\bar{X}$ 100	92.0	88.6	89.3	96.2
$S_{\bar{x}}$	1.2	1.7	2.0	1.5

* ate for 20 min

The relative uptake of injected I^{131} by the thyroid gland as calculated from mean, daily, pre-feeding neck counts and standard sample counts (means of 5 one-minute counts) is illustrated in Fig. 5. The pattern of I^{131} uptake in these sheep appears quite similar and shows that a peak in uptake was reached 6 to 7 days after I^{131} injection.

Changes in jugular blood-sample activity during eating are shown in Table 4. These results appear contrary to those expected with increased release of I^{131} as suggested in Table 3. To account for both decreased neck counts and decreased activity of venous blood sampled during eating, several factors must be considered. If I^{131} release into the thyroid vein was increased during eating, I^{131} levels in jugular venous samples would decrease if total blood flow through the jugular vein increased relatively more than I^{131} release into the thyroid vein.

Table 4

Mean cpm of jugular blood samples taken before, during, and after feeding expressed as per cent of mean pre-feeding cpm.

before feeding	during feeding (min)			after feeding
	0-10	11-20	21-30	
100	89.7	92.6	100.7	106.8
100	96.2	92.2	99.0	99.0
100	91.6	97.1	94.2	100.3
100	90.6	94.5	*	88.3
$\bar{X}$ 100	92.0	94.1	98.0	98.6
$S_{\bar{x}}$	1.4	1.1	1.9	3.8

* ate for 20 min

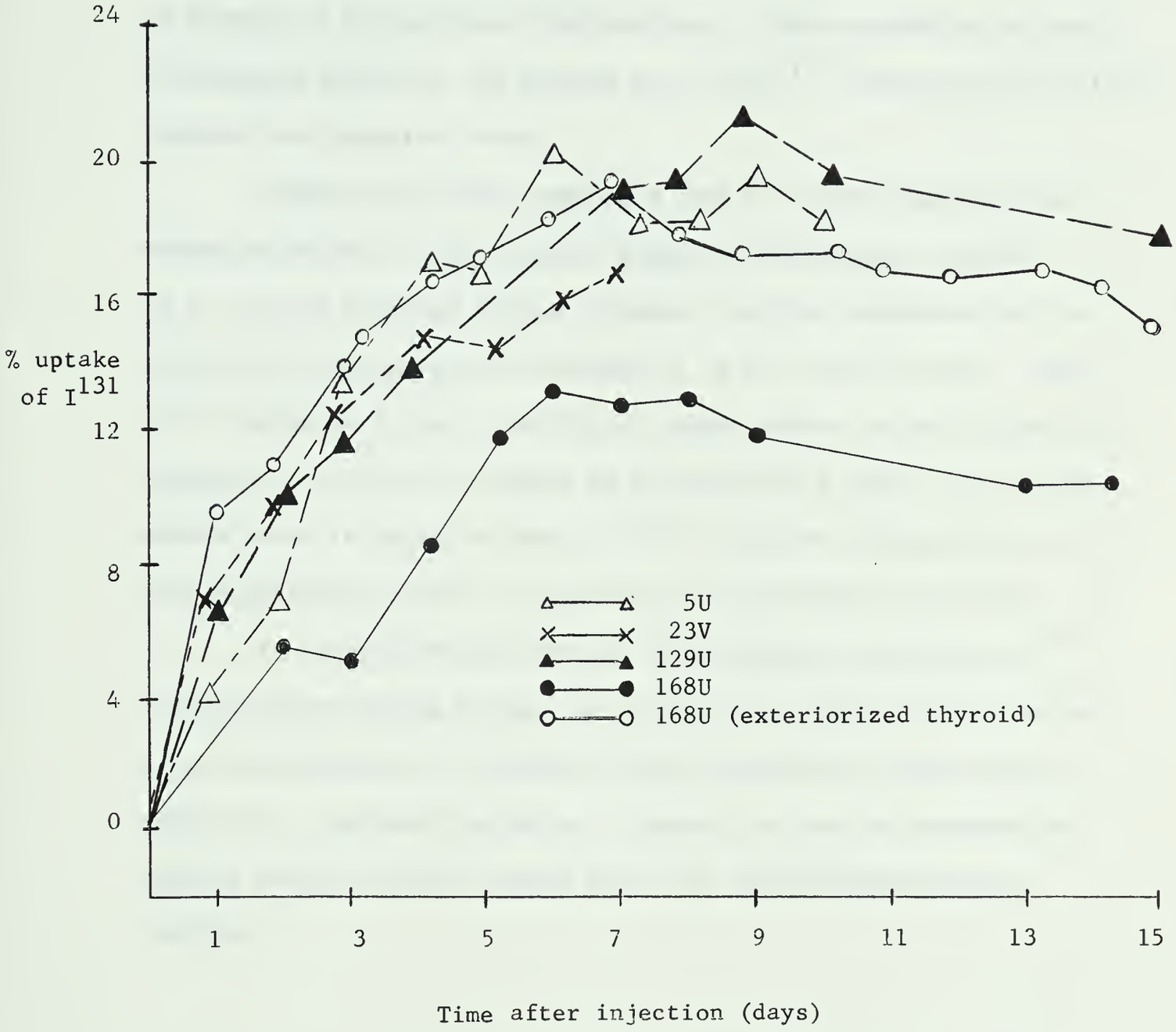


Figure 5. Relative uptake of injected I^{131} by the thyroid gland, calculated from mean daily pre-feeding neck counts and standard sample counts.

Another possibility is that release of I^{131} into the lymphatic system (Daniel, Pratt, et al., 1967) was enhanced by swallowing and by passage of boluses down the esophagus. This movement might exert a massaging effect on the thyroid and force I^{131} containing secretions through the lymphatic ducts.

Brown-Grant (1961) suggested that an iodide concentrating mechanism exists in the salivary glands. Subsequently, Harden et al. (1965) provided further evidence for this mechanism and concluded that it functions independently of the thyroid gland. They also demonstrated that in euthyroid humans almost as much iodide is secreted in saliva as is taken up by the thyroid gland. If similar events occur in sheep, release of I^{131} in saliva during eating may be a significant factor in accounting for decreased neck counts.

An alternative explanation of decreasing circulating I^{131} concentrations during eating, as reflected by jugular blood samples, is an increased rate of thyroid hormone degradation in the liver rather than decreased secretion. However the real occurrences may involve several factors, among which the aforementioned may be included.

Trial 3 Changes in thyroid gland activity during eating

Objective

Trial 3 was designed to measure the effect of the duration of eating on the release of I^{131} from the thyroid gland and to provide a comparison for the results of 30 min feeding sessions of Trial 2.

Experimental

Two wether sheep, 135T and 244T, were each given 300 μc I^{131} sc and were maintained on 1000 g alfalfa-brome hay per day. Feeding experiments commenced on the fifth day after injection and were continued for 6 days. The sheep were fed once daily and the tests were run in duplicate for 10, 20, or 30 min. Neck counts were recorded before, during, and after feeding, as in previous trials. After each test the sheep finished their daily ration.

Results and Discussion

The changes in neck counts recorded for sheep 135T and 244T in Table 5 are smaller than those recorded for sheep 5U and 23V in Table 3. Several factors may account for this difference. Sheep 135T and 244T were wethers and at least a year older than the rams. Because metabolic rate is stimulated by the male sex hormones and generally declines with age, the differences in thyroid I^{131} activity between the wethers and rams might be attributed to age and sex. However, the consistent drop in neck counts during eating would suggest that thyroid secretion was enhanced during eating.

Table 5

Changes in neck counts during 10, 20, and 30 min periods expressed as per cent of mean pre-feeding cpm.

day	sheep	pre-feeding	feeding 0-10	feeding 11-20	feeding 21-30	post-feeding
5	135T	100	98.0			101.6
8	135T	100	94.8			95.7
10	244T	100	87.3			94.3
7	244T	100	97.5			100.2
	$\bar{X}$	100	94.4			98.0
	$S_{\bar{x}}$		1.8			1.8
6	244T	100	94.8	93.4		94.6
9	244T	100	94.4	93.1		93.1
7	135T	100	98.1	97.8		96.4
6	135T	100	98.7	98.4		94.6
	$\bar{X}$	100	96.5	95.7		94.7
	$S_{\bar{x}}$		1.1	1.4		0.7
5	244T	100	98.7	97.1	94.1	95.9
8	244T	100	95.7	94.8	95.1	96.8
9	135T	100	97.3	98.7	99.7	101.8
14	135T	100	96.1	93.9	93.7	93.5
	$\bar{X}$	100	97.0	96.1	95.6	97.0
	$S_{\bar{x}}$		0.7	1.1	1.4	3.7

Trial 4 Measurement of thyroid blood I^{131} activity

Objective

The concentration of thyroid secretions in jugular venous blood is affected by the rate of thyroid secretion and the extent of dilution by jugular blood. As dilution is proportional to blood flow, changes in jugular blood flow under constant secretion rates would alter concentrations of thyroid secretions in jugular blood samples. Therefore, measurements of changes in jugular blood I^{131} activity may not represent only changes in thyroid secretion rates. Trial 4 was designed to measure I^{131} activity, of thyroid venous blood, from

sheep 168U.

Experimental and Results

The thyroid gland of sheep 168U was exteriorized according to the procedure developed by Falconer (1963). Thyroid gland activity was measured by I^{131} uptake (Fig. 5) and was similar to both the pre-operative rate and the rates obtained from the other sheep.

Labeled thyroid venous blood was sampled from the single loop containing the thyroid gland, jugular vein and carotid artery, through a retrograde catheter inserted into the jugular vein. Sampling from this preparation proved unsatisfactory for two reasons: jugular blood flow above the thyroid was difficult to stop during sampling; and considerable manipulation of the loop, and therefore the thyroid gland, occurred during sampling. The results of tests using this method are presented in Table 6a, but because of the procedures involved, it was considered that these may not reflect the physiological occurrences. Subsequently, after a second loop was made to enclose only the jugular vein above the thyroid gland (Fig. 1), more consistent values, as reflected by the decreased standard errors, were obtained for I^{131} activity in thyroid venous blood (Table 6b). Measurements of jugular I^{131} activity were also recorded.

Discussion

The contention that mechanical stimulation of the thyroid gland enhances release of iodinated compounds may also apply to the exteriorized thyroid preparation.

Table 6

Mean cpm of thyroid and jugular venous blood sampled before, during, and after feeding expressed as per cent of mean pre-feeding cpm.

a. Single loop

pre-feeding		feeding (10 min)		post-feeding	
thyroid	jugular	thyroid	jugular	thyroid	jugular
100	100(75.0)*	99.9	93.5(73.2)	106.1	98.0(77.5)
100	100(81.8)	95.9	91.4(74.8)	94.7	105.9(81.6)
$\bar{X}$ 100	100(78.4)	97.9	92.4(74.0)	100.4	102.0(79.6)

b. Double loop

100	100(59.8)	93.2	99.5(64.0)	95.1	99.5(62.7)
100	100(78.0)	96.7	80.3(66.1)	98.7	94.8(80.3)
100	100(80.5)	94.8	100.1(83.4)	92.2	114.0(93.0)
$\bar{X}$ 100	100(72.8)	94.9	93.3(71.2)	95.3	102.8(78.7)

* values in parentheses represent the ratio of jugular to the corresponding thyroid blood cpm.

In the event of lymph duct regeneration, drainage would probably lead to the jugular. Mechanical stimulation of the gland could enhance release of thyroid secretions, either via the thyroid vein, lymph duct(s) or both, but these changes should then be apparent in I^{131} measurements (Table 6a). Alternative explanations may account for the higher activity in samples obtained from the single loop during eating. To completely stop jugular blood flow during sampling, partial constriction of the carotid artery may have occurred. Under these circumstances, transient increases in thyroid arterial blood pressure may have enhanced wash-out of I^{131} from the gland. This possibility was definitely eliminated in the double loop preparation.

Although comparison of results in Table 4 and Table 6 indicates little difference in the changes in blood I^{131} activity during eating, a significant difference in isotope levels is seen between undiluted thyroid vein and diluted jugular vein blood samples.

Trial 5 Jugular blood flow

Objective

As previously explained, changes in jugular blood flow could markedly alter the jugular concentrations of thyroid effluents. When blood was sampled during eating, especially in trials involving the exteriorized thyroid, a consistent increase in venous return was visually apparent. Trial 5 was designed to measure this change in jugular blood flow.

Experimental

Trial 5 involved measurement of blood flow in each jugular of a sheep with an exteriorized thyroid (168U) and in one jugular vein of an intact sheep (23V) by the dye-dilution technique previously described.

Results and Discussion

The results in Table 7 indicate a nearly two-fold increase in jugular blood flow during the first five minutes of eating followed by a gradual decrease during the next 10 minutes. The tendency towards decreased blood flow after feeding, as compared to pre-feeding flow rates, may result from decreased plasma volume during eating

(Stacy and Brook, 1965; Brook and Blair-West, 1968; Christopherson, unpublished results). The pattern of flow through the exteriorized jugular during eating, was similar to that through the intact side.

Table 7

Jugular blood-flow rates measured by dye dilution and expressed as per cent of mean pre-feeding flow rate.

sheep	pre-feeding	feeding (min) 0-5	6-10	11-15	post-feeding
23V	100	184	197	174	74*
168U	**100	192	176	166	113
168U	100	197	176	146	79*
$\bar{X}$	100	191	183	162	89
$S_{\bar{x}}$		4.5	7.0	8.3	12.2

* post-feeding values estimated because hemolysis in blood samples
** this measurement made on exteriorized thyroid side

Trial 6 Thyroidectomy and thyroxine replacement therapy

Objective

An indication of the role of thyroid hormones in controlling metabolic and cardiac activity during eating should be obtained by regulating the amounts of thyroid hormones available to the sheep. This might be approximated by thyroid ablation, followed by thyroid hormone replacement therapy. Trial 6 was designed to measure the cardiac effects of thyroid hormone withdrawal and thyroxine replacement.

Experimental

Sheep 129U was thyroidectomized and thereafter given sufficient $CaCl_2$ in its drinking water to supply 10 mg Ca per 100 ml water. This procedure was thought necessary since the parathyroid glands were probably removed with the thyroid gland, although macroscopic examination of the thyroid lobes revealed no parathyroid tissue. After two months of calcium supplementation the decision was made to withhold $CaCl_2$ treatment because sufficient Ca should be available in the alfalfa hay used to maintain this animal. Careful observation of the sheep during the following months revealed no adverse symptoms. Thyroxine replacement (1 mg thyroxine/3 days) was commenced at 12 months and 20 months after thyroidectomy and maintained for 3 months in each instance.

Results and Discussion

The effects of thyroidectomy and thyroxine replacement therapy on heart–rate changes during eating were examined in Trial 6. Marked changes in appetite were noticed within three weeks of thyroidectomy. The intact animal would eat continuously for as long as 60 minutes, but after thyroidectomy, uninterrupted feeding would last for only 15 minutes. Periods of continuous feeding remained short until well after the completion of the first thyroxine replacement series. During this period of decreased appetite the animal appeared tense and excitable, tachycardia was more frequent and increases in heart rate of 20 beats per minute would occur during periods of restlessness. Twenty months after thyroidectomy, prior to starting the second thyroxine replacement series, eating was easily sustained for 30

minutes.

Table 8 shows the mean heart-rate responses before thyroidectomy, before both periods of T_4 replacement, and during the second T_4 replacement series. The heart-rate response to eating during these four periods appears quite uniform even though the pre-feeding rates differ by nearly 40 beats per minute. The possibility of hyperplasia of thyroid tissue,not removed by thyroidectomy, before the second T_4 replacement period is open to speculation, because 20 months after thyroidectomy, the heart-rate response during eating appeared quite similar to the normal response and absolute values were nearly identical. The ensuing T_4 supplementation increased resting heart rate by about 20 beats per minute but did not affect the response to feeding. The sheep appeared to have regained its stamina 20 months after thyroidectomy and would eat continuously for 30 minutes. Figure 6 provides a comparison of changes in heart rate before and after thyroidectomy, and illustrates the gradually diminishing heart rate response which occurred during the third and fourth week after thyroidectomy.

Table 8

Mean heart rates of sheep 129U, before and during feeding, calculated for 10 min periods (all means represent 2 or more runs).

	pre-feeding	feeding		
min.	10-0	0-10	11-20	21-30
before thyroidectomy**	51	73	87	105
9 months after	88	115	123*	
20 months after	56	71	100	95
during T_4 replacement	78	94	104	107

* ate for 15 min
** Hays, 1968

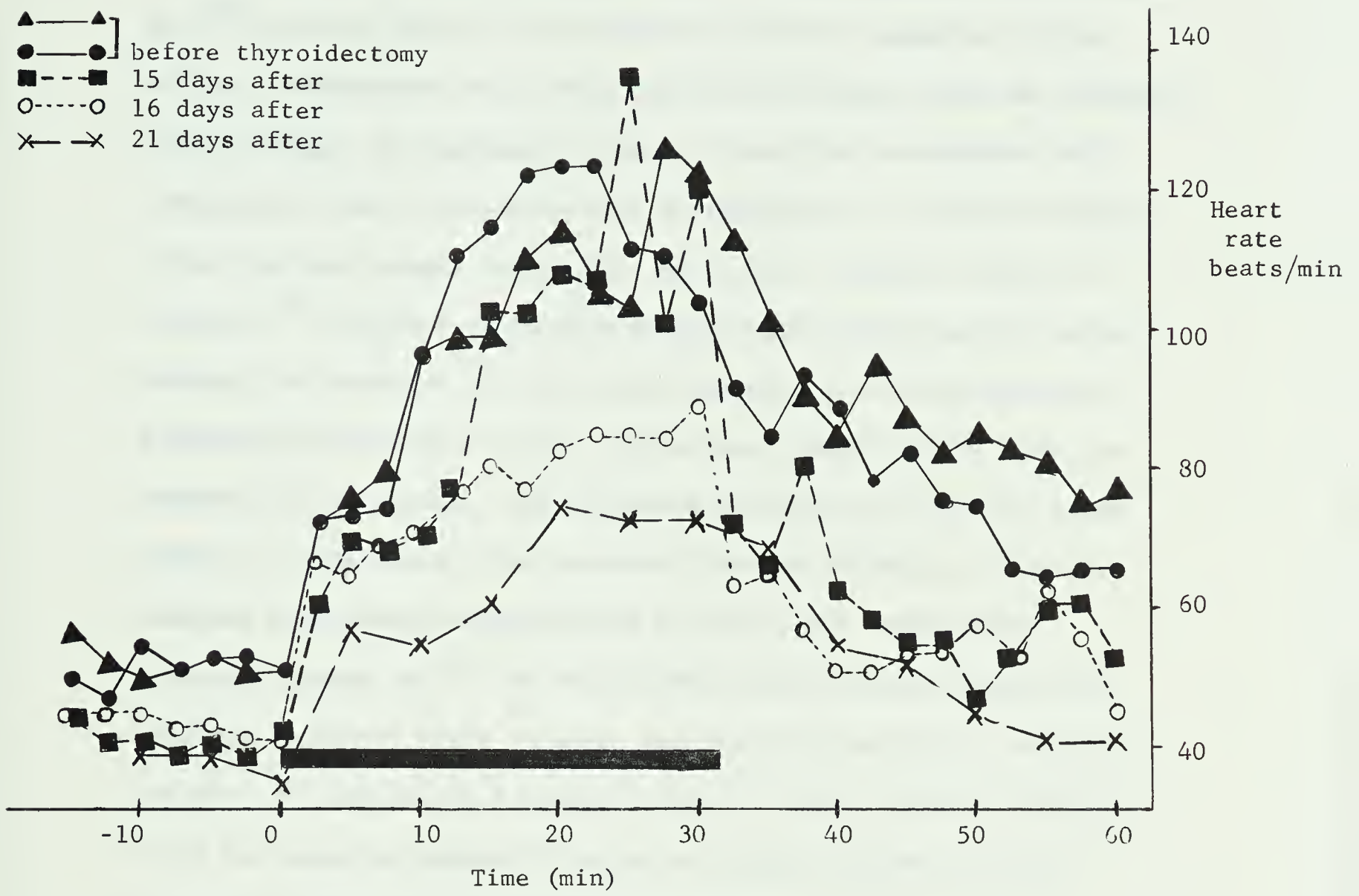
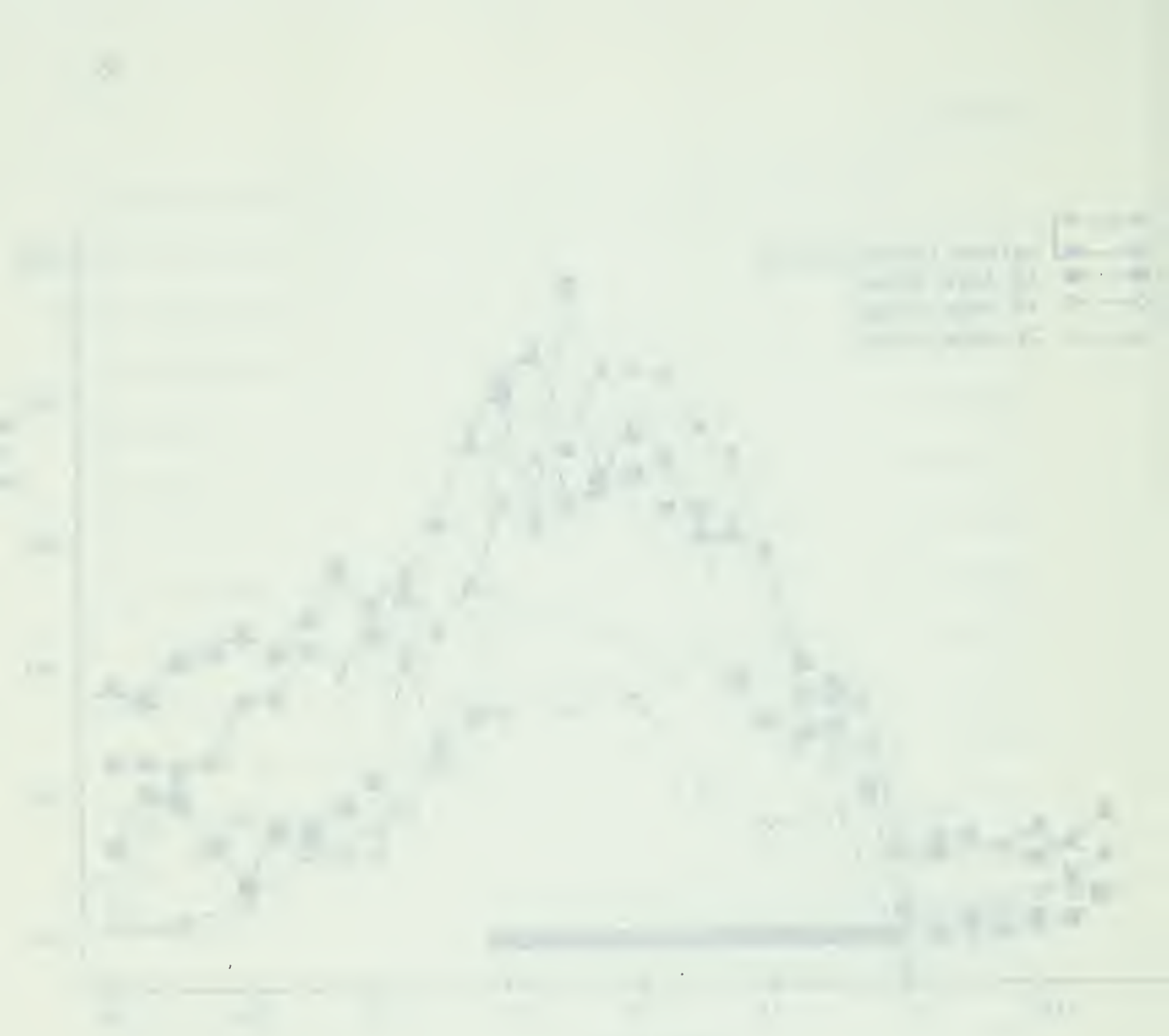


Figure 6. Mean heart rate responses of sheep 129U before thyroidectomy, before T_4 replacement, and during the second T_4 replacement series.

Solid bar - represents duration of eating



Discussion of Experiment I

From the results of Experiment 1, it is suggested that release of I^{131} from the region of the thyroid gland was augmented during eating. Furthermore, blood flow through the jugular vein was increased during eating. An increase in jugular blood flow concomitant with a decrease in neck counts might be an indication of increased thyroid blood flow and hormone secretion. One could, therefore, expect increased I^{131} activity in thyroid venous blood sampled during eating. However, the results of Trial 4 were contrary to this expectation. A decrease in activity in both jugular and thyroid venous blood was observed during eating. The decreased activity in systemic blood (Table 6) might result from increased systemic finding of thyroid hormones and increased degradation by the liver. Furthermore, increased uptake of I^{131} by the salivary glands during intensified salivary secretion might decrease jugular blood activity. Decreased activity of jugular blood sampled below the intact thyroid (Table 4) could be caused by greater dilution of thyroid effluents during increased venous return through the jugular.

Although measurements of arterio-venous differences in I^{131} activity might help to establish both the rates of thyroid secretion and salivary uptake during the aforementioned periods, the tendency for the proportional drop in jugular activity to be greater than that of thyroid blood (Table 6), would suggest enhanced thyroid secretion. Changes in thyroid blood dilution by fluctuations in jugular blood flow may, however, mask thyroid secretion.

It appears unlikely that the formation of iodinated protein in saliva (Weiss et al.,1962) influenced neck counts after labeling the sheep with I^{131}, or that increased release of saliva containing I^{131} during eating affected the change in neck counts when the collar was used because positioning of the probe was well controlled and radiation from the salivary glands was probably not counted by the detector.

In the last trial, thyroidectomy seemed to have little effect on the cardiac response of sheep 129U. However, conclusions cannot be drawn because marked changes in appetite occurred during the course of this trial. The possibility of hyperplasia of residual thyroid tissue should not be dismissed. The similarity of responses before thyroidectomy to those observed 20 months after surgery, is too great to remove this uncertainty.

C. Experiment II Changes in Blood Composition and Metabolic Activity Effected by Eating and Drug Treatment

A linear relationship between increases in heart rate and oxygen consumption during feeding has been demonstrated in ruminants (Webster 1966, Hays 1968). It has been suggested that the autonomic nervous system exerts at least partial control over cardiac activity in sheep during feeding. From the results of Experiment I, it appeared that thyroid gland secretion was enhanced during eating. From the magnitude of these metabolic responses, one could expect significant shifts in metabolite balances and blood distribution. This experiment was designed to measure the rate and direction of changes in blood composition during eating and to record the physiological effects of different drug and hormone treatments.

Trial 1 Respiratory exchange and venous glucose measurement

Objective

The first trial was designed to monitor the effects of eating on venous plasma-glucose levels and to measure the magnitude of changes in heart rate and oxygen consumption of two intact and one thyroidectomized sheep.

Experimental

Trial 1 consisted of 20 experiments: six with each of sheep 5U and 168U, and eight with sheep 129U which had been thyroidectomized nine months previously. The first three runs with each animal involved measurement of heart rate and oxygen consumption for 30 min before feeding, during 60 min of feeding, and for 30 min after feeding.

The animals had previously been fasted for 24 hours. The remaining tests included blood sampling at 5 min intervals before and after feeding for 30 min, and at 3 min intervals during 30 min of feeding. These samples were later analyzed for plasma glucose. Difficulty in obtaining consistent runs with sheep 129U was encountered because the animal ate for only short periods of time, ranging from 15 to 30 min.

Results and Discussion

The mean values of heart rate and oxygen consumption of sheep 5U and 168U appear in Table 9. Heart rate increased rapidly at the beginning of food consumption, reached peak values within 15 to 20 min, and generally declined as the pace of eating decreased. The rate of oxygen consumption increased more rapidly, reaching maximum values within 5 to 10 min. The magnitude of these changes was quite similar to those recorded by Webster (1966) and Hays (1968). The feeding response of the thyroidectomized sheep was similar, but of shorter duration. During the preparation of plasma samples in these trials, consistent differences in packed-cell volumes were observed in the centrifuged blood samples taken before and after the onset of eating. From the results of plasma analyses, a consistent trend in glucose concentrations for both the intact and thyroidectomized sheep was apparent. The graphs in Fig. 7 are merely representative of 24 runs in which venous glucose was scanned. There were differences between runs in initial blood glucose concentration, in the magnitude of changes, and in the intensity and duration of eating. It therefore seemed impractical and meaningless to combine these results for statistical analysis (see Appendix). Plasma glucose

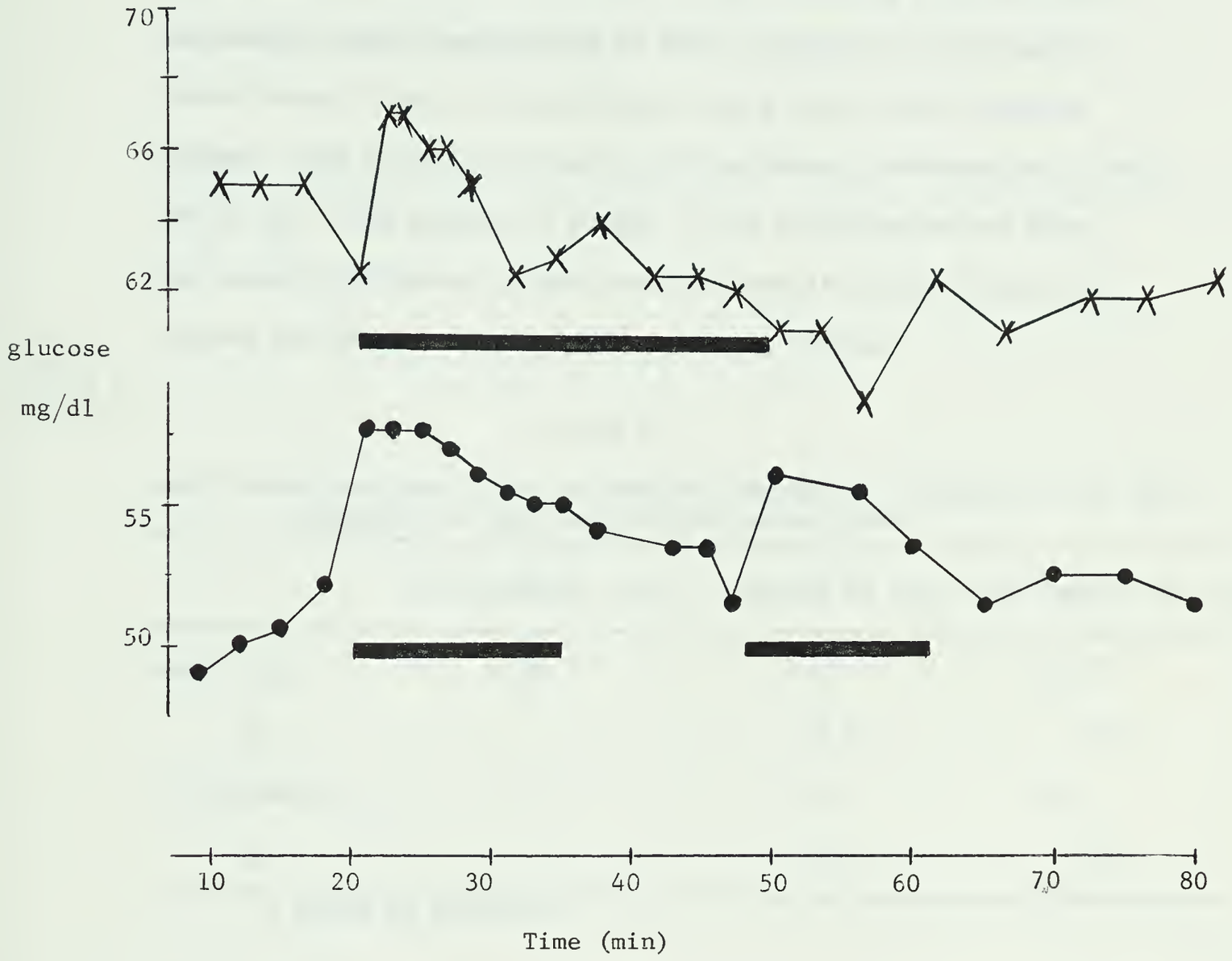


Figure 7. Measurements of plasma glucose concentrations during feeding.

Solid bar - represents duration of eating
X———X sheep 168U
0———0 sheep 129U (thyroidectomized)

levels usually increased about 5% over pre-feeding values within two minutes after presentation of feed, decreased to pre-feeding levels within 10 min and continued to drop slowly until feeding stopped. The duration of feeding during these blood-sampling trials was 30 min. The pattern of change in the thyroidectomized sheep was slightly different in that the increase in plasma glucose was greater and the decrease was slower during eating.

Table 9

Mean values for heart rate and oxygen consumption of sheep 5U and 168U expressed as per cent of pre-feeding mean*

	pre-feeding 30 min	feeding 60 min	post-feeding 30 min
heart rate	100	174	139
$S_{\bar{x}}$		5.8	6.7
O_2 consumption	100	142	114
$S_{\bar{x}}$		8.6	4.4

* means of six runs

The initial peak in venous glucose, which sometimes occurred one or two minutes before feeding, might have resulted from stimulated glycogen breakdown at the onset of eating, or even earlier, upon anticipation of eating. Alternatively, both glucose utilization and arterial concentrations might have remained constant while blood flow increased, thus causing an increase in venous glucose concentration. Notwithstanding the differences in rate and duration of eating, initial glucose levels and their magnitude of change during eating, the

general trend for decreasing venous plasma glucose concentrations persisted throughout the feeding period.

Trial 2 Estimation of plasma glucose, FFA, protein, PBI, and hematocrits

Objective

A trend for steadily decreasing venous glucose levels during eating and a sudden change in packed cell volume at the onset of eating were noted in Trial 1. Changes in jugular plasma-glucose levels do not indicate the extent of glucose utilization at any specific site. The second trial was designed to measure carotid and jugular concentrations of plasma glucose, free fatty acids (FFA), and protein to attempt to determine the contribution of increased muscular and secretory activities to elevated energy expenditure during eating. Measurement of jugular and venous plasma PBI concentrations was intended to supplement results of Experiment I. The study of the effects of beta-adrenergic blockade on arterial and venous hematocrits was included.

Experimental

Sheep 5U and 168U were used in eight experiments, which involved serial blood sampling and continuous measurement of heart rate before, during, and after eating. Feed was last offered 24 hrs before each experiment. The duration of feeding varied between 15 and 30 min because food was kept available only as long as the animals continued to eat with minimal interruptions. During these experiments, the sheep were held in an elevated metabolism crate and situated in full view of all sampling and recording equipment.

During the detailed study of hematocrit fluctuations in sheep 168U, 1.3 ml blood samples were drawn at 10 sec intervals for 2.5 min. A three-way tap and disposable syringe were used in the rapid sampling procedure. As each sample was drawn from the catheter, it was transferred to a plastic test tube through the outlet arm of the tap. Because the catheter held 1.3 ml blood and was not flushed between samples, a 10 sec delay occurred between sampling from the animal and dispensing that sample from the catheter.

Sheep 23V was used in the duplicate tests involving propranolol. Two control runs were performed during which heart rates and hematocrits were measured. Feed was offered for 30 min in the four tests. Propranolol was injected iv over 2 min (60 mg/30 ml), 30 min before the sheep was fed. Blood was sampled throughout the trial while a continuous recording of heart rate was made.

Results and Discussion

Because feeding periods were of unequal duration, the results of a typical run are presented in Fig. 8. Although the trends shown in these graphs were common to all runs, the rates of change in plasma concentrations of the different constituents varied slightly.

The decrease in arterio-venous glucose difference coincided with the peak in venous glucose concentration and probably resulted from the sudden increase in blood flow which masked the uptake of glucose across the head. The steady decrease of both arterial and venous concentrations during eating was more pronounced in runs in which the pre-feeding glucose levels were higher and in which the sheep ate more vigorously. Consequently, this decrease might be

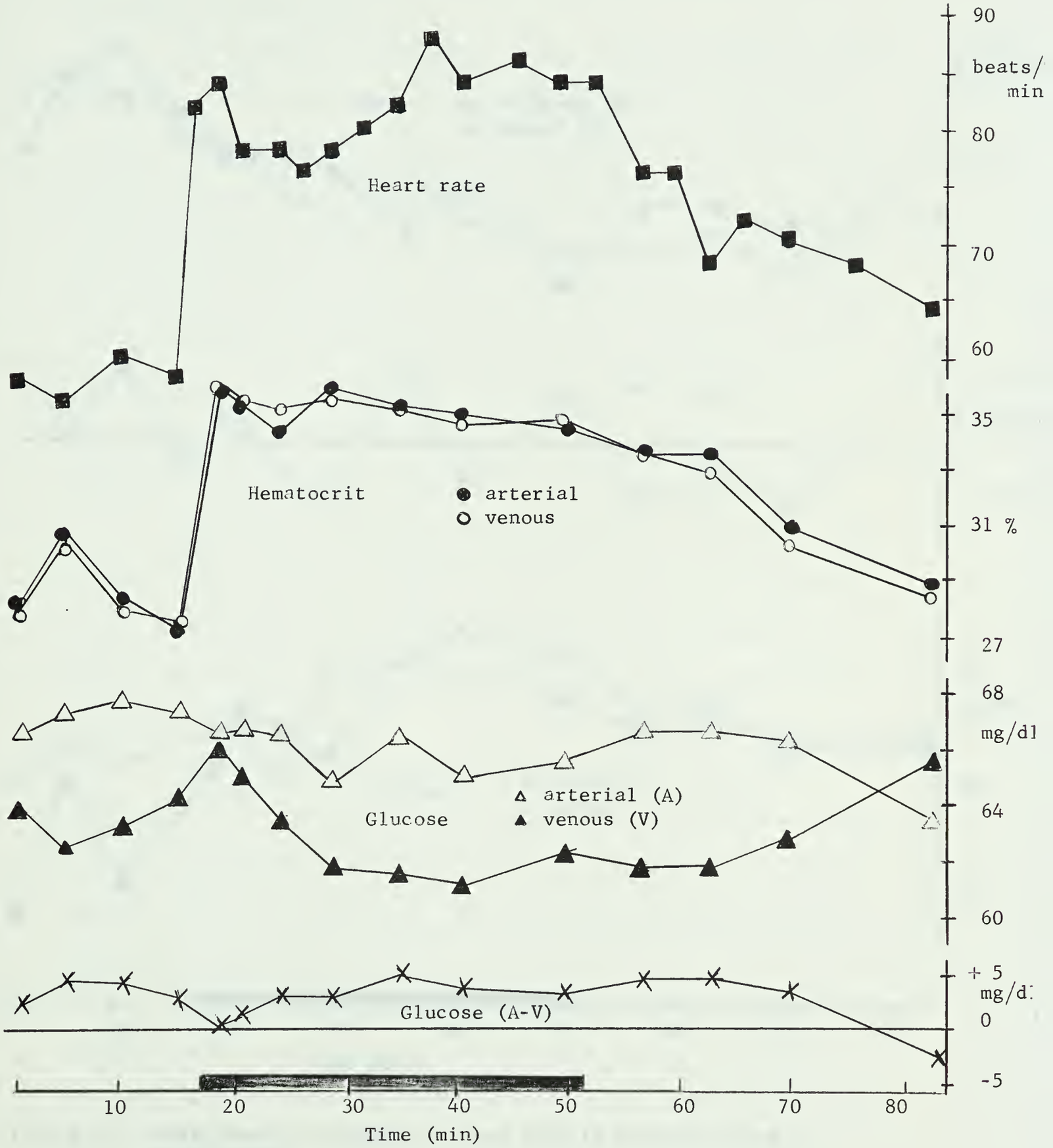


Figure 8a. Measurements of heart rate, hematocrit, and plasma glucose levels in sheep 168U.

Solid bar - represents duration of eating

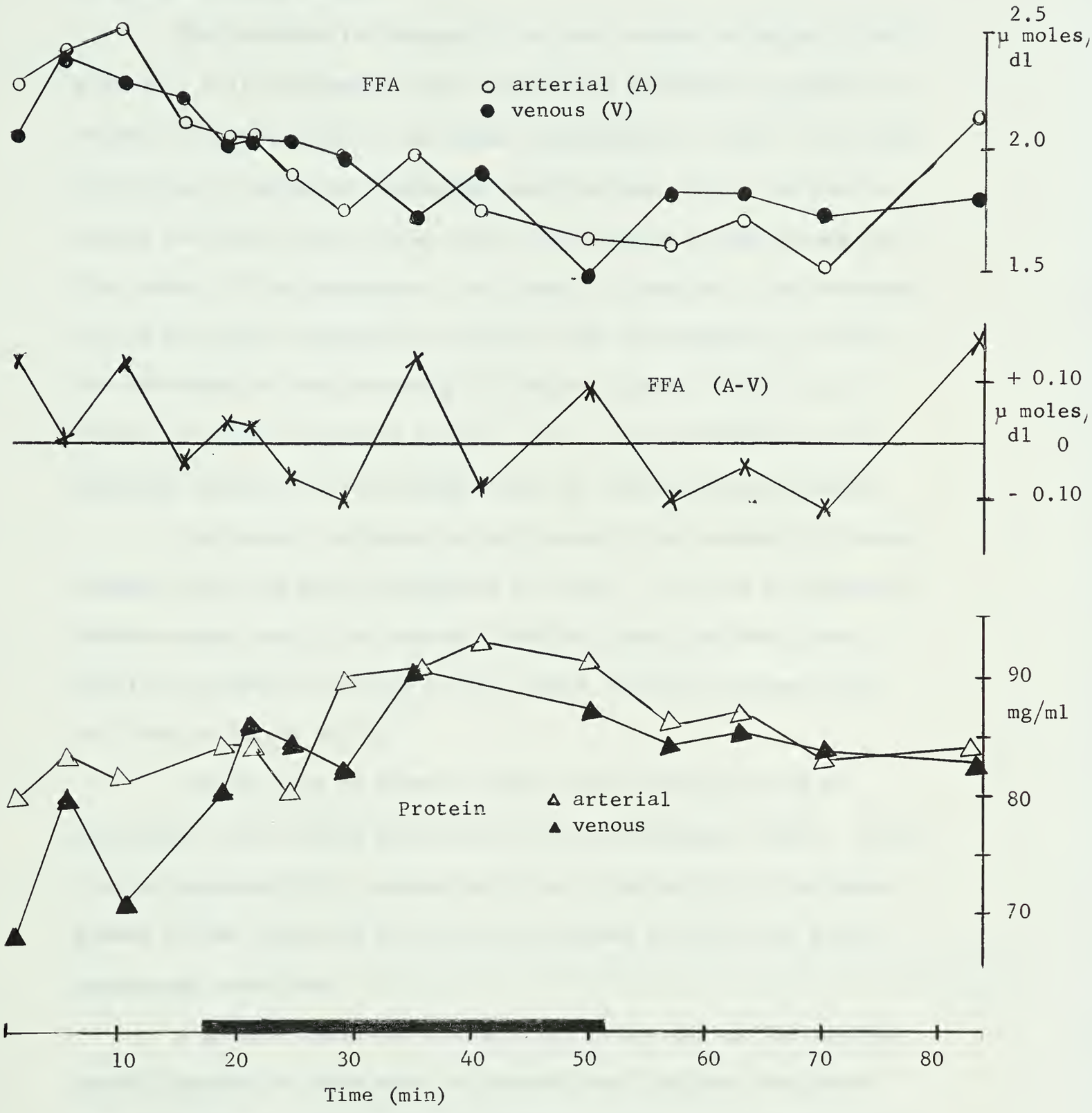


Figure 8b. Measurements of plasma FFA and protein concentrations in sheep 168U.

Solid bar - represents duration of eating

attributed to both increased muscular and secretory gland utilization of glucose during eating.

The increase in hematocrit was both sudden and significant. From Fig. 9 it is apparent that most of the increase in packed cell volume occurred within 30 sec after presentation of food. The more rapid rise in arterial hematocrit would indicate that a release of stored red blood cells, rather than a decrease in plasma volume, was the cause of this phenomenon. No change in hematocrit was observed for 30 min after propranolol injection and the response to eating was unchanged by beta-adrenergic blockade. These results are in accord with those reported by Ross (1967), who concluded that the emptying mechanism of the spleen does not involve beta-receptors.

The gradual decrease in both arterial and venous FFA levels suggests that FFA mobilization did not occur. In view of decreased plasma volume during the course of eating (Brook and Blair-West, 1968), the gradual decrease in FFA levels indicates increased FFA utilization during eating.

The 12% rise in plasma protein levels during eating is consistent with results published by Stacy and Warner (1966). This rise in plasma-protein concentration is an indication of decreased plasma volume resulting mainly from increased salivary and gastro-intestinal secretion.

A general trend for elevated PBI values during the feeding period appeared in five sets of thyroid blood analyses and three runs during which only jugular blood was sampled. The gradual rise in PBI concentration during eating was similar to the coincident

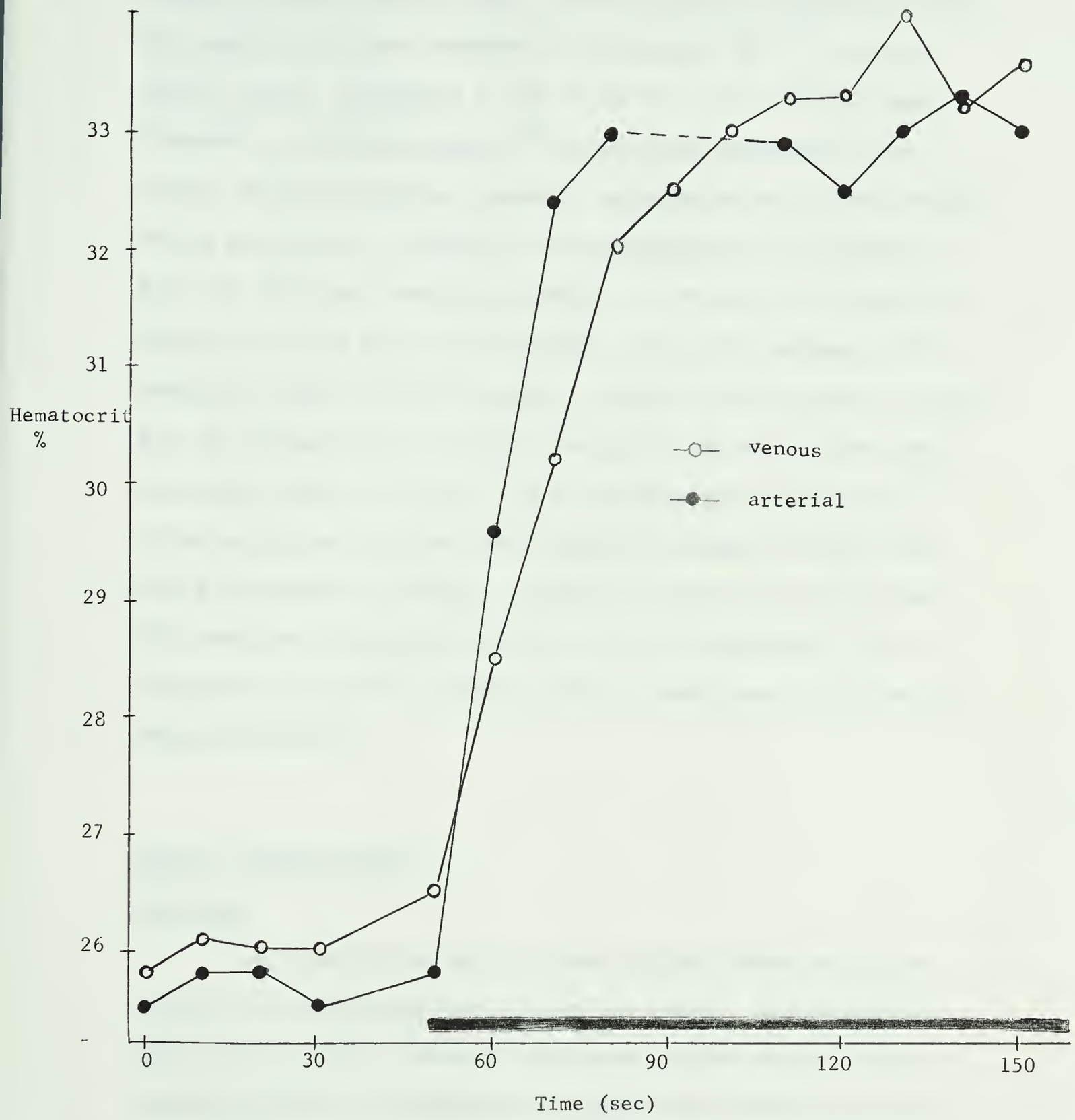


Figure 9. Detailed measurements of arterial and venous hematocrits at the start of feeding.

change in plasma protein values. Similar differences between thyroid and jugular blood were observed in measurements of I^{131} activity (Table 4 and 6, Experiment I) and estimates of PBI concentrations (Appendix). Although plasma I^{131} activity was decreased during eating, PBI concentrations tended to increase during the same period. During most tests, a decrease in PBI concentration was observed at both the onset and cessation of eating. In view of the increase in blood flow at the beginning of eating, the initial decrease in PBI levels may simply reflect dilution. In view of the increase in blood flow at the beginning of eating, the initial decrease in PBI levels may simply reflect dilution. The differences between PBI and I^{131} trends might have resulted from changes in inorganic iodide levels during the course of eating. Although the trend for rising plasma PBI levels was consistent with the results of Experiment I, the decrease in Plasma I^{131} activity cannot be explained on the basis of these measurements.

Trial 3 Plasma insulin

Objective

The trend for decreasing plasma glucose levels during the course of eating was apparent in previous trials. The purpose of Trial 3 was to study changes in peripheral plasma insulin levels to determine whether the hypoglycemic response was mediated by insulin.

Experimental

One feeding trial was conducted with sheep 5U, which had been fasted for 24 hrs. Jugular blood was sampled at 10 min intervals and

centrifuged, after which, the plasma samples were frozen for glucose and insulin assays.

Results and Discussion

The means of duplicate glucose determinations and triplicate insulin analyses appear in Fig. 10. The insulin levels are in the low to normal range for sheep. It appears unlikely that the hypoglycemic response to eating was mediated by insulin, as hormone levels several fold greater than those observed are required to produce hypoglycemia (Manns and Boda,1967). Therefore, it is debatable whether the increased insulin levels noted during eating were of any physiological significance. It must be realized, however, that these measurements represent peripheral insulin levels and do not necessarily reflect hepatic portal vein concentrations. It is possible that insulin levels in blood flowing to the liver were considerably higher and possibly of greater physiological significance.

Trial 4 Blood gas analyses

Objective

Preliminary investigations were designed to measure the latency and magnitude of changes in blood pH, pO_2, and pCO_2 after feed was offered.

Experimental

Jugular blood flow, pH, pO_2, and pCO_2 were measured before, during, and after a 15 min eating period in sheep 168U and 23V. Sheep 168U was used in one test during which simultaneous carotid

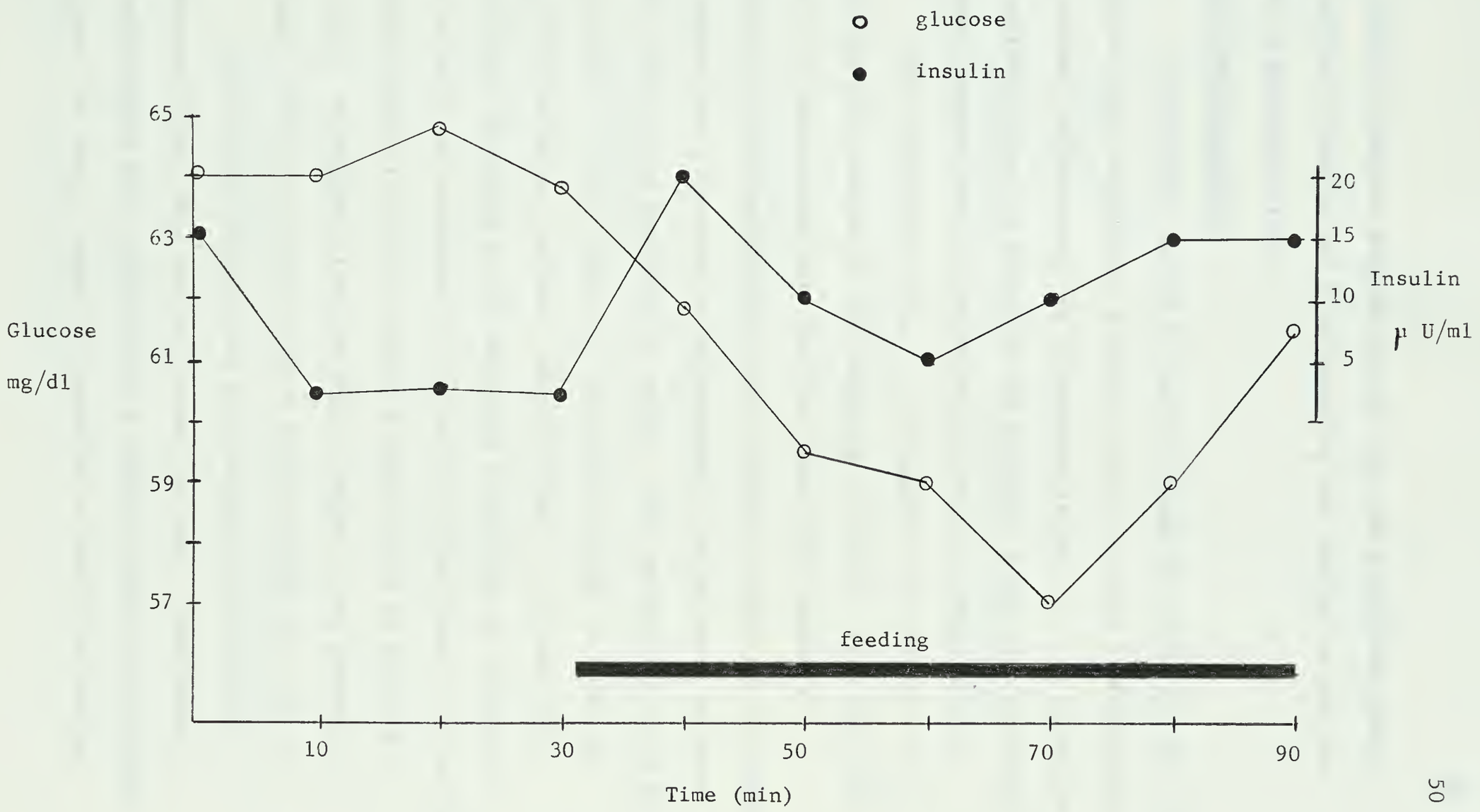


Figure 10. Mean jugular-plasma insulin and glucose levels in sheep 5U during feeding.

and jugular blood was sampled for pH, pO_2, and pCO_2 measurements. The method of feeding and blood sampling was similar to that outlined in previous trials.

Results and Discussion

An individual record of jugular plasma OD, blood pH, pO_2, and pCO_2, and heart rate during a feeding trial, in which bromsulphalein was infused intravenously, is illustrated in Fig. 11. As blood flow was estimated from the extent of dye dilution, blood flow rates were inversely proportional to the OD values (Trial 5, Experiment I).

From the graphs in Fig. 11, it appears that changes in all recordings were maximal at the beginning of eating. From the three sets of pH measurements obtained from sheep 168U (Appendix), a difference in pre-feeding values was indicated between the two sheep. It appears that a decrease of approximately 0.06 pH units occurred within 2 min of the beginning of eating. This decrease in blood pH was probably a direct result of the sudden increase in arterial pCO_2 (Christopherson, personal communications). If respiratory activity was decreased at the beginning of feeding, an increase in alveolar pCO_2 and consequently arterial pCO_2 might occur. In addition, a greater proportion of the available oxygen would be taken up by the blood. Subsequent tissue uptake of oxygen in the presence of decreased alveolar pO_2 could cause a lowered venous pO_2 (Fig. 11).

The blood analyses of one test revealed arterio-venous differences in pH, pO_2, and pCO_2 across the head. Unfortunately the measurements were confounded by a peculiar heart-rate response throughout the test. Sheep 168U exhibited a widely fluctuating

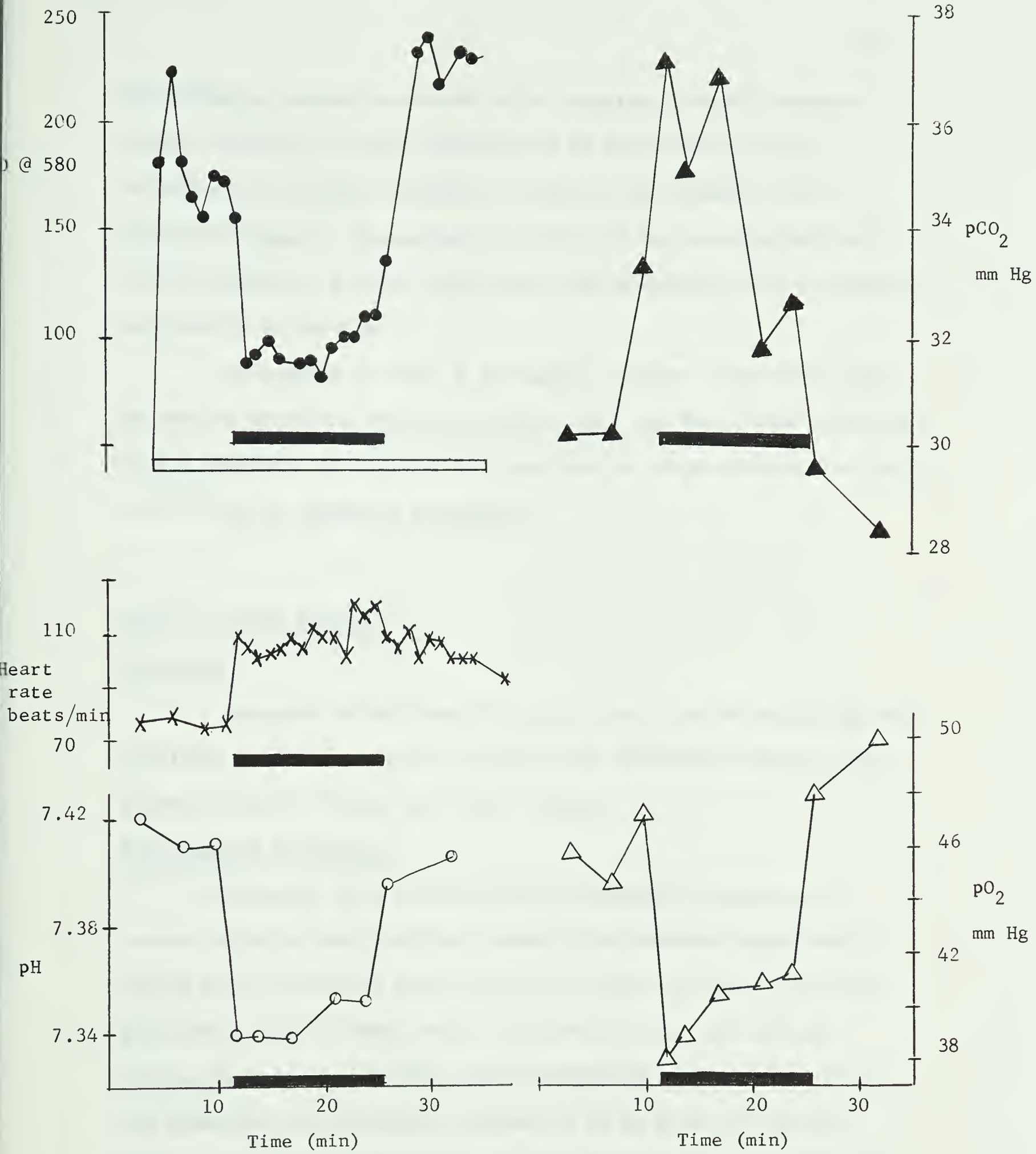


Figure 11. Individual record of heart rate, jugular-plasma OD, blood pH, pO_2, and pCO_2.

Solid bars - duration of eating
Open bar - duration of dye infusion

heart rate on several occasions while carrying a carotid catheter. Sudden increases in heart rate from 70 to 170 beats per minute accompanied by severe shivering occurred in the absence of any exogenous stimuli. Mechanical irritation of the inner carotid wall by the catheter tip might have caused some discomfort, but an adequate explanation is lacking.

The results of Trial 4 do suggest, however, that both venous pH and pO_2 decreased while blood flow, pCO_2, and heart rate increased at the beginning of eating. The magnitude of change decreased slightly as the rate of ingestion diminished.

Trial 5 Blood pressure

Objective

Increases in both heart rate and blood flow during eating were confirmed in previous trials. Trial 5 was designed to monitor blood pressure before, during, and after feeding.

Experimental and Results

Two sheep (135T and 244T) with permanently exteriorized carotid arteries were used for cursory blood pressure measurements. During one of the three runs, a 0.25 mg/kg dose of propranolol was given over 1 min to sheep 244T, 10 min before food was offered. During the two control runs, mean blood-pressure peaked when food was presented, and thereafter remained 10 mm Hg above pre-feeding pressure for 10 min, before dropping to original values. Under beta blockade, the initial peak in blood pressure was abolished while the response during the next 10 min was similar to the controls.

Discussion

Because the increases in heart rate and blood flow were much greater than the corresponding increase in blood pressure during the first 10 min of eating, some other adjustments in the cardiovascular system occurred. An overall decrease in peripheral resistance seems a reasonable explanation for this combination of results.

Trial 6 Parasympathetic blockade with atropine sulfate

Objective

The implication of parasympathetic control over heart rate during eating was made by Hays (1968) on the basis of feeding trials conducted with sheep having pharmacologically denervated hearts. The suggestion was made that pharmacological blockade of autonomic control of the sheep's heart was effective for two hours following atropine (0.05 mg/kg) and propranolol (0.5 mg/kg) infusions. However, cursory experiments indicated that atropine induced tachycardia may last for only 15 to 30 min, the duration of blockade depending upon the dose and rate of atropine administration.

Trial 6 was designed to estimate the duration of atropine-induced parasympathetic blockade.

Experimental and Results

Continuous recordings of heart rate and reticulo-rumen motility were obtained from sheep Gus in four tests. Due to the complex nature of rumen contractions it was difficult to determine their frequency. However, the diphasic peaks of reticular contractions (Duncan, 1951) were easily identified and are presented in Table 10.

Within two minutes of starting atropine administration (0.05 mg/kg infused over 15 min through jugular catheter), reticulo-rumen motility ceased. After another 20 minutes contractions approached their normal rate and intensity. The effects of atropine on heart rate, illustrated in Fig. 12, lasted about 15 min after infusion stopped. The rate of food ingestion was markedly decreased during atropine infusion and seemed to reflect the degree of reticulo-rumen activity.

Table 10

Rates of reticular contraction before, during, and after feeding expressed as contractions per 30 min.

run	pre-feeding	feeding		post-feeding
		0-30	31-60 (min)	
1	20	73	53	37
2	21	75	60	30
3	28	68		36
$\bar{X}$	23	72	56	34

In the four tests conducted with sheep 23V (Fig. 13, 14, 15) atropine blockade of the parasympathetic system was challenged with periodic injections of acetylcholine (Ach). Cardiac arrest lasted approximately 4 sec following iv injection of 6 mg Ach (0.05 mg/kg) into the conscious sheep (Fig. 14). This dose appeared rather severe as the animal nearly collapsed. After 30 min the heart was stopped for 6 sec with 3 mg Ach. Although the next dose of Ach was further reduced to 1 mg, heart contraction stopped for 5 sec. However, after

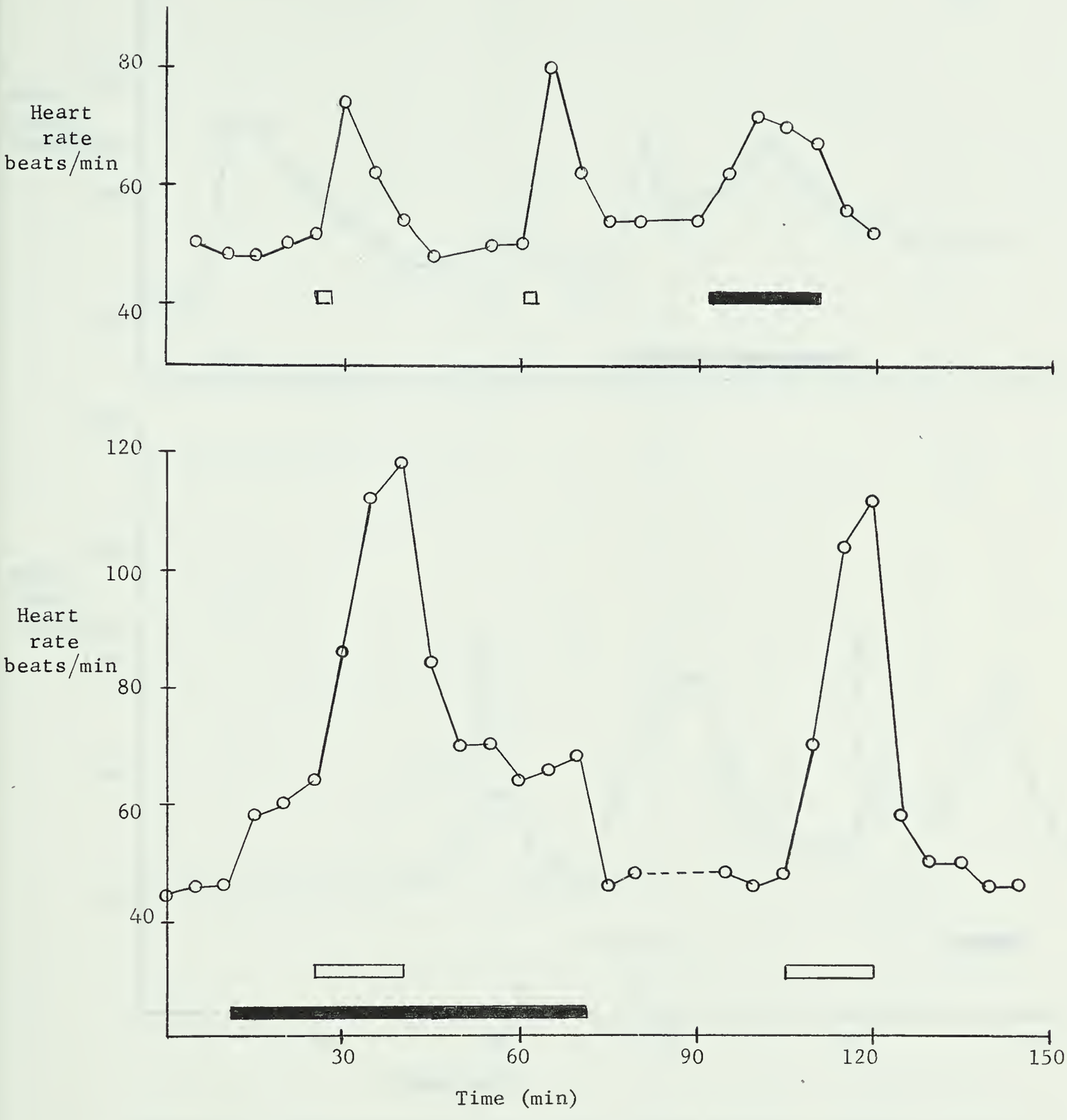


Figure 12. Measurements of heart rate from sheep 168U during feeding and atropine infusion.

Open bar - 0.05 mg/kg atropine infusion
Solid bar - duration of eating

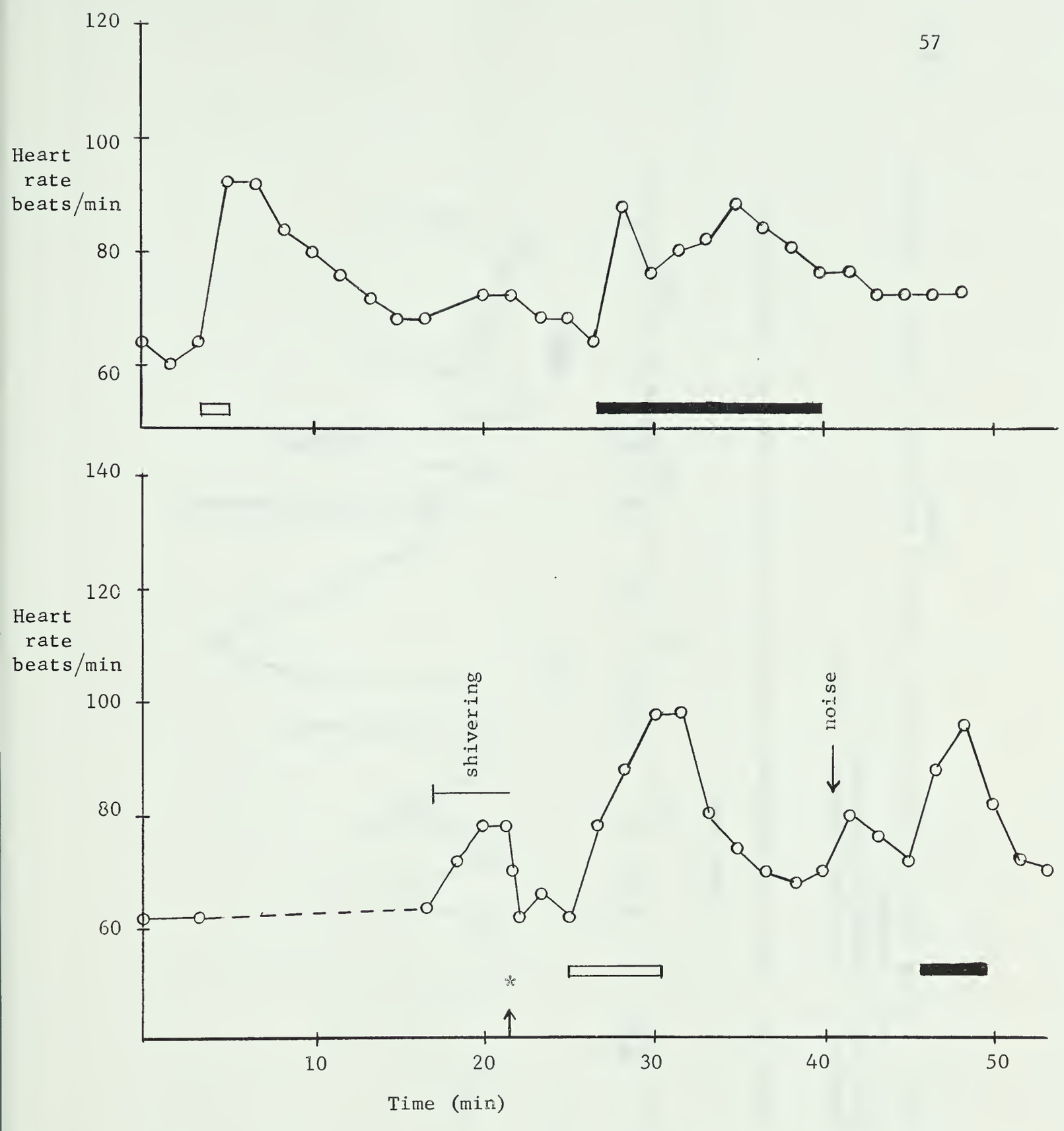


Figure 13. Measurements of heart rate during feeding, atropine infusion, and acetylcholine injection.

Open bar - 0.05 mg/kg atropine infusion
Solid bar - duration of eating
* - heart stopped
arrow - 1 mg acetylcholine injection

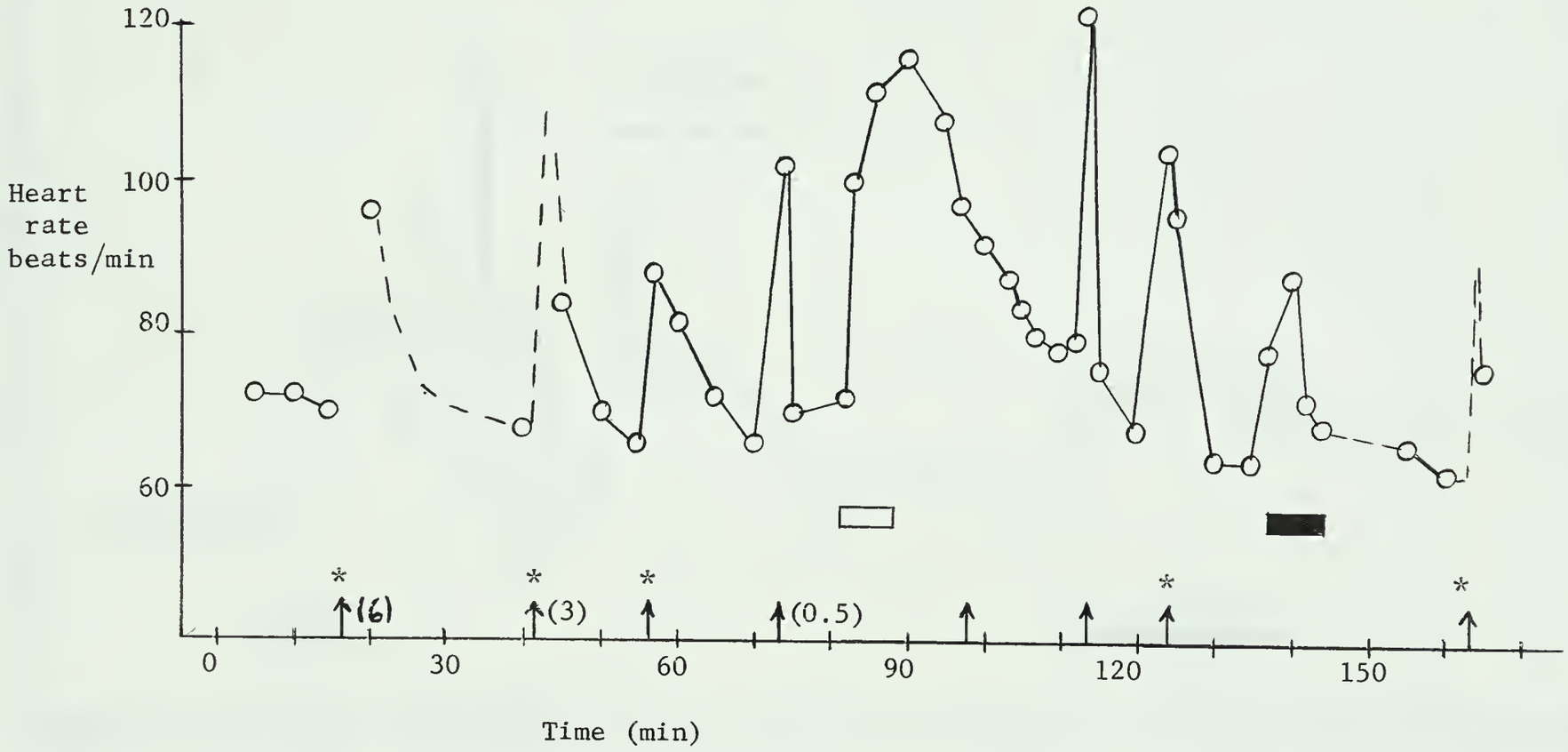


Figure 14. Heart-rate response of sheep 23V to various doses of acetylcholine.

Open bar - 0.1 mg/kg atropine infusion
Solid bar - duration of eating
* - heart stopped
arrow - 1 mg acetylcholine injection (values in parentheses indicate different doses)

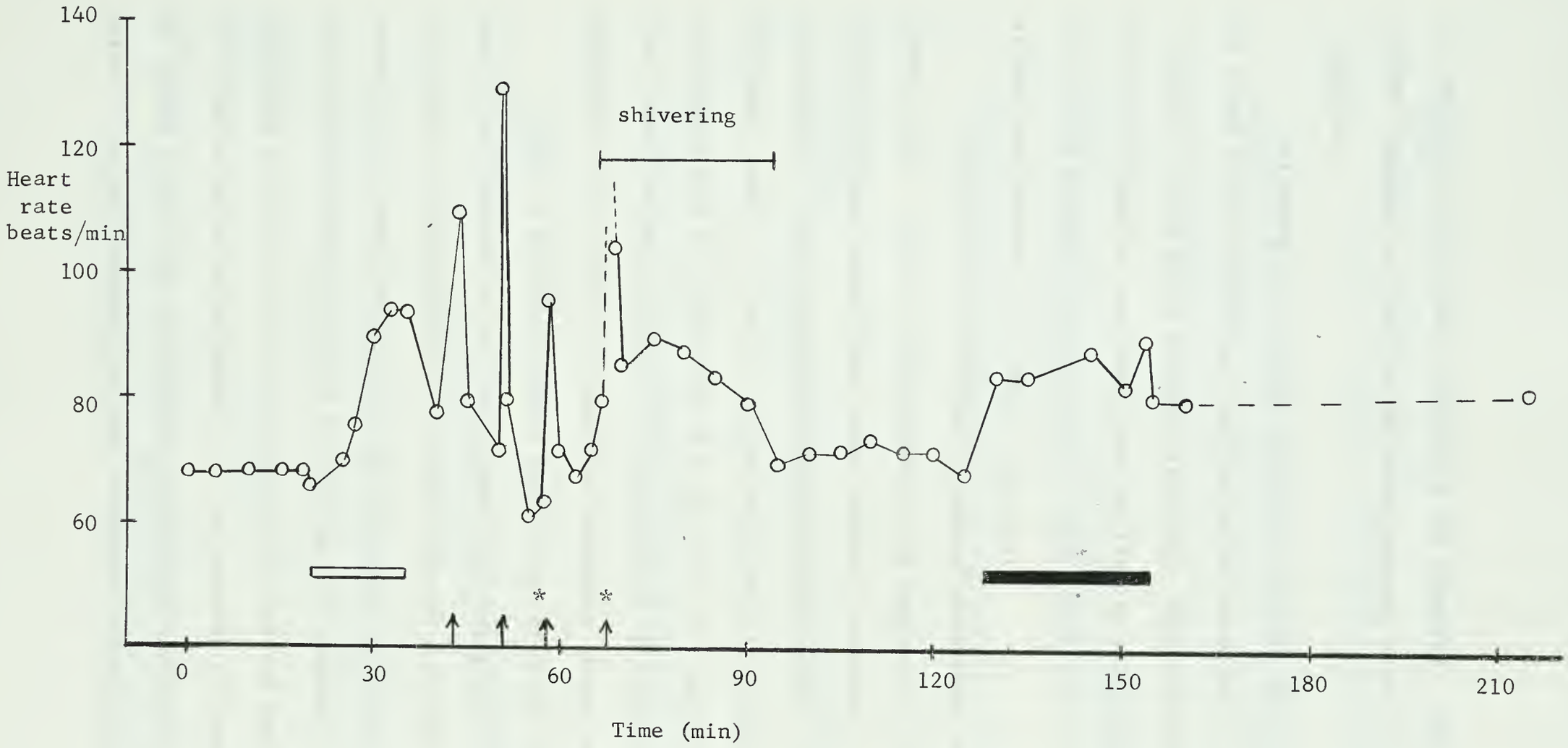


Figure 15. Heart-rate response of sheep 23V to 1 mg injections of acetylcholine after 0.05 mg/kg atropine infusion.

Open bar - 0.05 mg/kg atropine infusion
Solid bar - duration of eating
* - heart stopped
arrow - 1 mg acetylcholine injection

0.5 mg Ach, only slight tachycardia was observed. This indicated a reflex response to the drop in blood pressure associated with Ach injection.

Following a 0.1 mg/kg atropine infusion, Ach was injected at 5 and 10 min intervals. Cardiac arrest occurred in response to 1 mg Ach, 20 min after atropine infusion. Parasympathetic stimulation sufficient to stop heart contraction was achieved 22 min after 0.05 mg/kg atropine infusion over 15 min. Tachycardia in response to Ach was observed 7 min after infusion.

The ninth test involved a terminal experiment with one Lincoln ewe. Carotid catheterization and exposure of the right vagal trunk was accomplished under Nembutal anesthesia. Continuous recordings of blood pressure and heart rate were made. Parasympathetic blockade, after 0.05 and 0.1 mg/kg infusions of atropine, was challenged with 0.2 mg/kg Ach (8.4 mg) at 5, 15, and 30 min intervals. This procedure was repeated after propranolol (0.5 mg/kg) and atropine (at two levels) blockade of autonomic nerves to the heart.

Fig. 16 provides graphic description of blood pressure and heart-rate response to Ach injections after atropine infusion of 0.05 mg/kg. Resting heart rate under Nembutal anesthesia remained steady at 138 beats per min and was unchanged by atropine infusion. After combined administration of atropine and propranolol heart rate decreased to 96 beats per min. Mean blood pressure seemed unchanged by either atropine or propranolol. The duration of atropine blockade, as judged by the lack of response following Ach injection, appeared longer after 0.1 mg/kg infusion, and was, therefore, considered dose dependent.

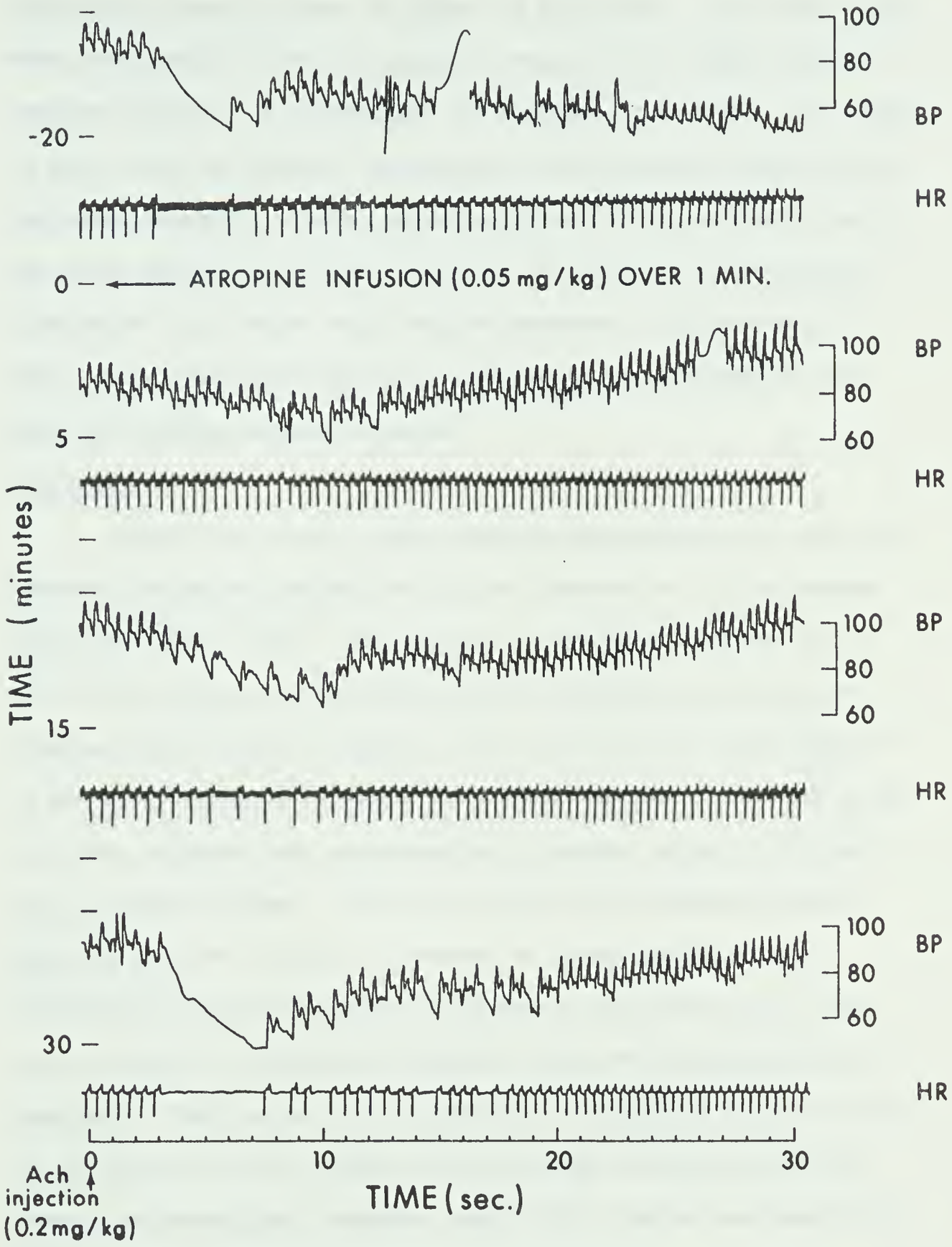


Figure 16. Individual record of blood pressure (BP - in mm Hg) and heart rate (HR) of an anesthetized sheep showing responses to injections of acetyl-choline (Ach) before and after atropine infusion.

Propranolol seemed to have no effect on Ach action. The right vagus nerve was severed in the mid-cervical region 70 min after the last combined infusion of propranolol and atropine (0.1 mg/kg). No change in heart rate was observed during nerve disection but blood pressure decreased slightly. Electrical stimulation of the peripheral end of the vagus induced bradycardia only for the duration of stimulation. Stimulation 5 min after vagal section produced a 30% decrease in heart rate. This same degree of bradycardia was effected 40 min after 0.05 mg/kg atropine infusion.

Discussion

Results of control tests involving measurements of reticular contractions agree favorably with those reported by Christopherson (1967) and Dukes (1955). The revival of reticular contractions 20 min after atropine infusion indicated that complete parasympathetic blockade was no longer effective. The ability to induce bradycardia 15 min after atropine infusion, and cardiac arrest 5 to 15 min later with Ach, suggests that parasympathetic receptor sites in the heart were no longer blocked. There was no doubt that pharmacological doses of Ach were injected. Blockade of parasympathetic nerve transmission during Ach induced bradycardia might have been effective because the injected Ach probably acted on the parasympathetic receptors. The absence of any heart-rate response to vagal disection 70 min after the last atropine infusion might have been caused by reduced parasympathetic receptor sensitivity during continued drug applications. Prolonged action of atropine might have contributed to the reduced efficacy of nerve conduction, which is illustrated

by a decrease in heart rate of only 30% during electrical stimulation of the peripheral end of the right vagus. Duncan (1951) suggested that the extreme regularity of reticular contractions was a response to rhythmic central discharges. The results of Trial 6 demonstrated revival of reticular activity within 20 min after atropine infusion, effects of injected Ach 15 min after infusion; and support the conclusion that atropine induced parasympathetic blockade in sheep remained effective for only 15 to 20 min. The magnitude and duration of partial inhibition was dose dependent.

Trial 7 Hormone infusions

Objective

As the possibility of hormonal control of physiological changes during eating had not been excluded, Trial 7 was designed to record short term metabolic and cardiovascular effects produced by infusions of various hormones in efforts to mimic the eating response.

Experimental

Simultaneous recordings of heart rate, blood pressure, rectal temperature, and respiratory exchange were obtained from sheep 135T during four trials involving iv administration of noradrenaline, TSH, T_3, and one involving ACTH. Sheep 244T was used once for corticosterone and ACTH injections.

Results and Discussion

No significant changes were recorded in response to infusions of TSH, ACTH, corticosterone, all at 1 μg/kg per min; or T_3, at 0.1 μg/kg per min. Sheep 244T did not survive the night following the trial in which a corticosterone dose of 5 μg/kg iv was rapidly injected. This dose produced slight fluctuations in blood pressure, but otherwise, seemed to have no effects.

Typical pressor responses to noradrenaline infusions of 0.1 to 1.0 μg/kg per min were observed. While blood pressure rose quickly, the initial cardiac response was a decreased rate of contraction. With the higher dose of noradrenaline, however, severe tachycardia developed very rapidly. Consequently the infusion was interrupted and thereafter continued at the lower dosage.

Although the dosage, route of administration, and source of hormone preparation might have been sub-optimal or unsuitable, the results of Trial 7 favor the conclusion that the aforementioned hormones do not initiate tachycardia and do not regulate metabolic rate during eating.

Discussion of Experiment II

The changes in blood composition and metabolic activity recorded in sheep during eating were direct consequences of the physiological processes involved in prehension, salivation, mastication, deglutition, and gastro-intestinal motility. The decreases in plasma glucose and FFA concentrations probably resulted from increased muscular and secretory gland energy utilization during the

aforementioned processes. The fluid shifts from plasma to the digestive tract, mainly through saliva and gastric secretions (Bailey, 1961; Stacey and Warner, 1967), resulted in apparent concentration of most plasma constituents, especially the plasma proteins. Hematocrits increased very rapidly at the beginning of feeding, apparently in response to splenic contractions and expulsion of red blood cells. Although peripheral insulin concentrations increased,the magnitude of change seemed insufficient to cause the general hypoglycemic trend during feeding.

The slight elevations in blood pressure during the first 10 min of eating were unaffected by beta-adrenergic blockade and appeared to be a consequence of the changes in heart rate, blood flow, blood volume and peripheral resistance. The initial peak in blood pressure, which was abolished by propranolol, may be attributed to sympathetic control. It is comparable to the blood pressure response observed in dogs (Fronek, 1968) during 2 to 3 min feeding periods.

Increased energy expenditure during eating stimulated elevated oxygen consumption. However, due to the delay in respiratory clearance of excess CO_2 at the onset of eating, blood pCO_2 increased and caused a drop in blood pH. The attenuation of arterio-venous pO_2 differences across the head* resulted from increased oxygen consumption presumably by the masticatory muscles and salivary glands.

Implications of Trial 6 support the presence of an atropine-degrading enzyme in sheep, as in goats, (Miller, 1966). The involvement of parasympathetic control during eating was demonstrated in feeding trials during which heart rate and reticulo-rumen motility

* see Appendix

was recorded. Although the extent of parasympathetic control over heart rate during eating was not determined, its involvement in stimulating tachycardia was suggested.

SUMMARY AND CONCLUSIONS

The involvement of the sympathetic and parasympathetic systems in the regulation of physiological changes in sheep during eating was demonstrated. While the rise in blood pressure in response to eating was diminished during beta-adrenergic blockade, the increase in hematocrit was unaffected. During atropine-induced parasympathetic blockade, tachycardia was observed and reticulo-rumen motility was inhibited. Blockade by atropine was effective for only 20 min.

Measurements of the magnitude and duration of changes in blood constituents and in hemodynamics, indicated the degree of adjustment which was necessary to maintain homeostasis. Rapid increases in blood flow and hematocrit were recorded at the onset of eating. Measurements of plasma protein levels indicated increased concentration during eating, apparently a consequence of decreased plasma volume. Concentrations of plasma glucose and FFA decreased during eating while PBI levels appeared to rise. Further evidence for enhanced secretion by the thyroid gland during eating was obtained from isotope trials.

The mechanism for accelerating metabolic rate during eating was not determined and results of various hormone treatments indicated no short-term effects on metabolic rate. However, thyroid secretions, classical metabolic stimulators, might stimulate oxygen consumption during eating. Nathanielsz (1967) reported evidence for faster disappearance of labeled thyroxine from blood during periods when animals were fed; when feed was withheld, either during

the day or night, rates of label disappearance decreased.

It seems possible that tissue uptake of thyroid hormones is regulated by critical threshold levels and that oxygen consumption is stimulated once the hormones are assimilated. The physiological adjustments which occur during eating might be essential for enhanced tissue uptake of these hormones. The role of TSH could be one of maintaining thyroid hormone concentrations near the threshold level whereas eating might cause levels to rise above threshold. These mechanisms could influence resting metabolic rate. Measurements of fasting metabolism in sheep demonstrated a progressive decline in metabolic rate (Marston, 1948; Blaxter, 1962; Young, 1968). Lack of thyroid secretions, normally produced during eating, might partially account for this progressive decline. Because thyroid hormones are in part responsible for regulating metabolic rate, the above hypotheses might be tenable.

The intricacy of changes which occur during eating and continue into the post-prandial period shows that eating generates a myriad of stimuli which influence the entire animal. The significant increase in oxygen consumption, the sudden changes in blood flow and distribution, and the complexity of metabolic shifts which occur during feeding, appear conducive to synthetic metabolism. The enhancement of growth hormone secretion during sleep (Honda et al., 1969) and the stimulation of thyroid secretion during feeding support the hypothesis that growth is periodic and that eating stimulates synthesis.

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APPENDIX I

Estimations of Jugular Plasma Glucose

135T 54(-34), 45(-24), 53.5(-14), 51(-4), 52.5(7), 48(16), 52.5(26), 49(36), 49(46), 52.5(56), 47(66), 52.5(76)
135T 32.7(-31), 34.1(-21), 35.3(-11), 35.8(-1), 31.1(10), 31.1(20), 32.7(30), 35.0(40) - based on whole blood an-
135T 74.5(-16), 74.5(-9), 73(-3), 73(2), 74(4), 72(7), 70(11), 72(18)‡ 69.5(29), 68(37), 69.5(49), 67(59). alyses.

244T 48(-40), 55(-30), 68(-15), replaced catheter, 83(-5), 80(5), 71(15),77(24),71(35),66(47),71(55),75(65),65(75).
244T 44(-31), 38.5(-21), 40.5(-11),42(-1),37.5(10),36(20),35(29),32(39),36.5(48),38.5(59),36.5(69),36.5(79).

5U 63(-21),64.5(-13),66.5(-10),67(-7),67.5(-1),72(2),68.5(5),67.5(9),64.5(12),64(16),62.5(19),62.5(23),58(27),
58(31),54.5(37),53.5(40),**,55(44),56(47),58.5(53),56(59),57(64).
5U 67.5(18),59.3(12),57.5(-5),57.5(-1),61.0(2),62.5(5),60.5(8),62.0(11),61.5(14),60(17),61(20),58(24),57.5(28),
58.5(33),57.5(36),58(42),59.5(47),58.5(52),58.5(58),59.0(64),59(68),**,58.5(72),57.5(77),57.5(82),58.5(87),
59.0(92),60(99),61(104),62.5(112),63(117).
5U 65.5(-24),59.5(-15),59.5(-5),60.5(-1),62.5(3),61.5(5),61(7),61.5(9),60(11),60.5(14),60(16),60(18),60(20),
60(22),58.5(25),58.5(28),60(32),60(35),59(42),**,58.5(46),6.15(50),61.5(56),61.5(64),65(69),63.5(79),61.5(80).

129U 53(-29),54(-24),55(-16),56(-8),57(-4),57(-1),58(1),59(4),58(6),59(8),59(10),59(11),58(14),57(16),**,58(18),
57(20),57(25),56(30),56(35),56(40),58(45),58(50),58(55).
129U 49.5(-16),49.0(-11),50.0(-8),50.5(-5),52.0(-2),57.6(1),57.6(3),57.6(5),57.0(7),56.0(9),55.5(11),55.0(13),
55.0(15),**,54.0(17),53.5(-6).
129U 53.5(-6),53.5(-4),51.5(-2),56.0(1),55.5(7),53.5(11),51.5(16),52.5(21),52.5(26),51.5(31),53.5(37),53.5(41).

168U 73(-18),70(-13),72(-8),68(-3),73(2),73(5),65(8),65(11),62(13),**,60(17),60(22),58(27),60(32),65(37),68(42).
168U 67(-21),65(-11),65(-8),65(-5),62(-1),67(1),67(2),66(4),66(5),65(7),62(10),63(13),64(16),62(20),62(23),62(26),
**,61(29),61(32),60(35),62(40),61(45),62(51),62(55),62(60),61(65),59(67),61(69),60(72),62(74),61(77)ruminating.
168U 64.0(-15),62.6(-11),63.4(-6),64.4(-1),66.2(2),65.1(4),63.4(8),61.8(12),61.2(24),62.3(33)‡ 61.8(40),61.8(46),
62.8(53),65.6(66).
168U 76(-18),77(-12),75(-6),75(-1),76(2),73(5),73(8),72(14),70(19),71.5(30),**,66.5(37),68.5(44),66(52),64(66).
168U 70(-15),68.5(-10),70(-5),71(4),70.5(6),69(9),66(11),63.5(19),65(25),66(35),**,63.5(40),61.5(56),63.5(71).

23V 98(-17),98(-12),97.5(-1),90(-2),97.5(1),94(4),94(7),94(10),94(13),90(20),89(27),90(32),90(37),92(42).

** Feed removed
Glucose concentrations in mg/dl

Values in parentheses indicate time of blood sampling relative to feeding

APPENDIX I CONTINUED

168U 57.0*(-32),56.5(-24),57.5(-20),55.5(-17),57.0*(-11),57.5(-8),58.0(-2),58.8(1),58.0(4),57.5(7), 56.2*(8), **,56.2(11),56.2(14),57(17),57.5(20),57.5*(23),57.5(26),57.0(29),58.0(32).

168U 50*(-21),51(-12),52(-8),54(-4),53(-1),52(3),52*(7),51(8),48(11),48(16),48(20),**,46*(22),50(25), 50(29),50(33),50(36),51*(37).

168U 43.0*(-23),47.5(-17),48.3(-13),48.0(-9),48.4(-3),47.2*(-1),48.2(3),49.5(7),45.3(11),49.8(16),44.5*(18), **,48.2(22),48.5(27),50.2(32),49.8(36),49.9(42),49.7*(43).

168U 66*(-17),69(-13),67(-10),67.5(-6),67*(-4),69.5(2),66(5),66(8),62*(10),62.5(14),60.5(17),63(19), 60*(21),66(23),64(26),62(30),58.5*(31),60.5(34).

168U 56*(-17),52(-12),50*(-8),50*(-6),51(-4),52(-1),55(2),55(5),53(8),53.5*(9),54.5(12),51.5(20),52(22), 51(26),53.5(30),46.5*(37),50(39),**,48.5*(53),52(55),52(62),51(69),51*(73).

168U 53.5*(-16),55(-13),50*(-10),52(-7),52*(-5),57(-2),58(1),57.5(3),55.5(5),54.5*(7),55.5(9),52(11),55.5(14), 55*(16),55(19),52(25),53(30),**,55*(34),56(36),58.5(44),58.5(50),59*(54),60(59),57*(62).

168U 58.5*(-40),61(-32),68.5(-15),68.5*(-7),66(-2),70(2),69*(15),7.5(17),70(21),69.5*(22),69*(28),71.5(29), 70*(32),74(34),72(38),71.5*(39).

* Jugular plasma samples

** Feed removed

Glucose concentrations in mg/dl

Values in parentheses indicate time of blood sampling relative to feeding

APPENDIX II

Arterio-Venous Measurements of Hematocrit, Glucose, and FFA.

1. H	A	28.3(-15),	30.8(-11),	28.4(-6),	27.3(-1),	35.8(2),	35.2(4),	34.3(8),	35.8(12),	35.2(18),	34.9(24),	34.4(33),	**,	33.5(40),	33.5(46),	30.9(53),	28.8(66)
	V	28.0	30.3	28.0	27.5	36.0	35.4	35.0	35.5	35.0	34.5	34.7	**	33.5	32.8	30.2	28.4
G	A	66.0	67.4	67.8	67.4	66.6	66.8	66.6	64.8	66.6	65.0	65.6	**	66.6	66.6	66.2	63.4
	V	64.0	62.6	63.4	64.4	66.2	65.1	63.4	61.8	61.6	61.2	62.3	**	61.8	61.8	62.8	65.6
F	A	2.30	2.44	2.54	2.14	2.08	2.08	1.92	1.76	2.00	1.76	1.64	**	1.62	1.74	1.50	2.16
	V	2.06	2.42	2.30	2.24	2.04	2.06	2.06	1.98	1.74	1.94	1.48	**	1.84	1.84	1.74	1.82
2. H	A	27.6(-18),	27.2(-12),	26.4(-6),	26.0(-1),	36.6(2),	35.3(5),	34.0(8),	34.0(14),	34.0(19),	33.1(25),	31.5(30),	**,	31.6(37),	29.2(44),	28.6(52),	26.6(66)
	V	27.2	27.1	26.3	25.4	36.2	35.0	34.5	34.3	34.0	33.0	32.3	**	30.8	29.1	28.5	26.5
G	A	78	76	77.5	76	76	78	78	75.5	76	76	73	**	72	71	71	67
	V	76	77	75	75	76	73	73	72	70	71.5	71.5	**	66.5	68.5	66	64
F	A	2.56	1.96	2.56	2.74	2.70	2.44	1.00	1.74	1.66	1.44	1.66	**	1.56	1.74	1.60	1.44
	V	2.70	3.20	2.50	2.44	2.70	2.28	2.48	1.94	2.56	2.40	2.36	**	2.61	2.48	2.92	2.16
3. H	A	25.6(-15),	27.0(-10),	26.7(-5),	27.0(4),	26.9(6),	33.8(9),	32.4(11),	35.5(19),	31.5(25),	29.0(35),	**	32.8(40),	27.5(56),	(71)		
	V	29.3	27.3	26.7	34.1	32.8	31.5	33.8	34.8	31.9	30.0	**	32.6				
G	A	71	71	71	71	70.5	71	69	68	68	67	**	66	66	65.5		
	V	70	68.5	70	70	70.5	69	66	63.5	65	66	**	63.5	61.5	63.5		
4. H	A	31.0(-20),	29.3(-15),	30.0(-10),	29.1(-5),	36.0(1),	36.0(5),	35.0(7),	36.0(11),	35.7(18),	33.7(23),	**	34.0(30),	29.6(45),	29.0(51)		
	V	30.6	30.3	29.9	29.5	35.9	36.1	35.0	35.2	34.8	33.0	**	34.7	29.2	28.8		

H - Hematocrit G - Glucose F - Free fatty acids A - Arterial

V - Venous ** - Feed removed Values in parentheses indicate time of blood sampling relative to feeding

APPENDIX III

Estimations of Thyroid Venous Plasma PBI

(1) 2.60(-21)*, 3.20(-12), 4.10(-8), 4.32(-4), 4.32(-1), 4.78(3), 3.00(7)*, 4.10(8), 4.55(11), 5.27(16), 4.32(20), **, 4.10(22)*, 3.45(25), 3.70(29), 3.97(33), 3.83(36).

(2) 6.1(-17), 6.1(-13), 5.0(-9), 6.4(-3), 4.1(-1)*, 7.0(3), 6.2(7), 7.5(11), 6.2(16), 4.8(18), **, 6.0(22), 6.5(27), 6.5(32), 5.9(36), 6.1(42).

(3) 2.75(-17)*, 3.00(-13), 2.90(-10), 5.70(-6), 1.05*(-4), 1.80(2), 2.40(5), 2.30(8), 2.10*(10), 2.90(14), 3.00(17), **, 2.80(19), 2.10*(21), 2.10(23), 3.25(26), 2.10(30), 2.40(31), 1.80*(34).

(4) 2.30(-17)*, 2.00(-12), 1.25(-8)*, 2.15(-6)*, 2.10(-4), 2.47(-1), 2.47(2), 2.10(5), 2.80(8), 3.80(9), 2.62(12)*, 2.97(14), 3.10(16), 2.97(20), 2.97(22), 3.55(26), 2.97(30), 2.47(37)*, 2.47(39), **, 2.80(53)*, 2.30(55), 3.10(62), 2.47(69), 2.15(73)*.

(5) 1.50(-16)*, 1.30(-13), 1.30(-10)*, 1.90(-5)*, 1.90(-2)*, 2.05(1), 2.40(3), 2.05(5), 1.70(7)*, 2.40(9), 2.22(11), 2.22(14), 1.70(16)*, 2.40(25), 2.22(30), **, 1.70(34)*, 2.40(36), 1.50(44), 2.05(50), 1.60(54)*, 2.05(59), 1.05(62)*.

(6) 3.2(-21), 4.1(-13), 4.1(-10), 3.8(-7), 3.8(-1), 3.6(2), 3.8(5), 3.7(9), 3.0(12), 3.0(16), 3.6(19), 3.4(23), 3.6(27), 3.6(31), 3.6(37), 3.6(40), **, 3.3(44), 3.4(47), 3.3(53), 3.3(59), 3.3(64).

(7) 3.0(-18), 3.3(-12), 3.3(-9), 3.3(-5), 3.0(-1), 3.0(2), 3.7(5), 3.5(8), 3.5(11), 3.3(14), 3.3(17), 3.5(20), 3.2(24), 3.3(28), 3.5(33), 3.5(36), 3.3(39), 3.3(42), 3.6(47), 3.6(52), 3.7(58), 3.7(64), **, 3.7(68), 3.6(72), 3.5(77), 3.6(82), 3.3(87), 3.8(92), 3.7(99), 3.7(104), 3.7(112), 3.6(117).

(8) 3.2(-34), 3.4(-25), 3.6(-5), 3.5(-1), 3.4(3), 3.3(5), 3.4(7), 3.6(9), 3.5(11), 3.5(14), 3.5(16), 3.6(18), 3.4(20), 3.8(22), 3.6(28), 3.4(32), 3.1(35), 3.5(42), **, 2.8(46), 2.8(50), 3.4(56), 3.6(64), 3.6(69), 3.6(75), 3.6(80).

* jugular plasma samples
** stopped eating
All values expressed as µg/dl plasma

Values in parentheses indicate time of blood sampling relative to feeding

APPENDIX IV

Arterio-Venous pH, pO_2, pCO_2 and OD

1. pH V 7.452(-8),7.460(-4),7.468(-1),7.452(2),7.381(5),7.407(8),7.418(10),7.413(13),7.408(15),7.424(18),7.434(21),7.423(30)

   pO_2 V 43.7 44.6 43.1 44.2 38.6 40.6 41.5 44.2 41.2 46.2 41.1 45.7

   OD .250(-5),.263(-4),.275(-3),.330(-2),.280(-1),.155(1),.170(2),.135(3),.120(4),.115(6),.138(7),.178(8),.178(9),.140(11), .150(12),.205(13),.228(14),.229(15),.262(16),.210(17),.227(18),.322h(19),.284(20),.250(21)

2. pH V 7.466(-7),7.465(-3),7.468(-1),7.406(2),7.435(7),7.421(10),7.433(13),7.432(16),7.438(19),7.446(22),7.447(27)

   OD .038(-7),.117(-6),.159(-5),.162(-4),.177(-3),.179(-2),.240h(-1),.115(1),.095h(2),.080(3),.090(4),.080(5),.108(6),.103(7), .146h(8),.092(9),.080(10),.140h(11),.118(12),.119(13),.110(14),.149h(15),.149h(16),.222h(18),.250h(19),.267h(20),.270h(21), .265(21)

3. pH V 7.420(-9),7.410(-4),7.411(-2),7.338(1),7.339(3),7.338(6),7.353(10),7.352(13),7.396(15),7.406(18)

   pO_2 V 45.8 44.6 47.2 38.0 39.0 40.5 40.8 41.2 48.0 50.0

   pCO_2 V 30.2 30.2 33.4 37.2 35.1 36.9 31.8 32.7 29.6 28.4

   OD .180(-7),.222(-6),.180(-5),.165(-4),.156(-3),.175(-2),.172(-1),.155(1),.088(2),.092(3),.099(4),.090(5),.158h(6),.087(7), .090(8),.081(9),.095h(10),.100h(11),.100h(12),.111h(13),.110h(14),.135(15),.233(18),.240(19),.218(20),.260h(21),.232(20), .288h(23)

4. pH A 7.498(-14),7.510(-9),7.503(-4),7.468(5),7.446(10),7.439(15),7.448(20), **,7.476(26),7.466(31),7.472(38),7.476(46),7.529(62)

   V 7.471 7.466 7.466 7.410 7.398 7.403 7.432 ** 7.420 7.410 7.410 7.416 7.454

   pO_2A 69.3 71.4 70.5 65.8 73.2 74.5 75.8 **79.5 93.8 71.8 75.0 72.2

   V 43.9 42.0 41.6 38.1 37.2 36.8 42.0 **40.8 43.2 42.8 43.4 34.4

   pCO_2 A 19.6 16.8 22.2 23.6 25.0 26.8 25.2 **24.2 25.8 32.2 24.7 21.4

   V 21.0 22.2 26.8 29.0 27.6 28.2 26.7 **29.2 30.6 28.4 26.5 29.0

A - Arterial V - Venous h - Hemolyzed plasma samples ** - Feed removed

Values in parentheses indicate time of blood sampling relative to feeding